

VIII- II -1. Project Research

Project 8

PR8 Project Research on the New Applicant Development Using the Characteristics of the Particles from the Neutron Capture Reaction

Y. Kinashi

Research Reactor Institute, Kyoto University

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 with 10ppm BPA, at 0.2Gy/min or at 0.04Gy/min without BPA. The frequency of mutations after neutron irradiation at 0.2Gy/min with 10ppm BPA was two times and more increased than that at 0.04 Gy/min without 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 biological effects, i.e., DNA damages, after the irradiation with heavy ion particles from BNC reaction. The induction of DNA double strand breaks (DNA-dsb) was detected by using the focus assay of repair protein. The Chinese hamster ovary cells (CHO/K1) and xrs-5, were irradiated at the KUR irradiation field for BNCT. 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. cells were assayed for conven-

tional colony formation, and DNA double strand breaks (DSBs) were detected by immune-staining using gamma-H2AX and 53BP1 antibodies. The focus assay used for detecting the DNA double strand breaks showed that the number of gamma-H2AX and 53BP1 foci 1hr post-irradiation were similar for the mixed radiation and the reference gamma-ray. 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. The mean of the foci sizes was not significantly different between the cell irradiated with gamma-rays and neutron, but the size distribution seemed to be larger in those irradiated with neutron.

PRS-3 tried to detect the BNCR condition for leaf tissue culture and callus conditions. To determine the effectiveness of BNCR for plant mutagenesis at irradiation of dry seed, leaf disc and callus stages, haploid and diploid tobacco (*Nicotiana tabacum* L.cv. Red Russian) plants were used because of its high callus induction, regeneration and totipotency. Tobacco callus is quite sensitive to BNCR, and there is little protection of nuclear proteins during active somatic cell division and having weak single strand of DNA. There was no difference of BNCR sensitivity between haploid and diploid tobacco plants. They found out the optimum BNCR conditions in tobacco leaf discs, which were 400, 600 or 800µM BPA and 30 or 60 minutes irradiations for expecting the maximum mutation rate. This result indicates that the leaf disc of tobacco plants seem not to absorb enough amounts of BPA into the cells for overnight treatment.

PRS-4 developed boron tracedrugs with their "on demand" traceability and investigated their physical force for neutron dynamic therapy (NDT). The curcuminoid boron trace drugs UTX-51 was used for neutron dynamic therapy. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS -PAGE) was performed to detect the decomposition by thermal neutron irradiation of human serum Gc protein treated with UTX-51. They found that the all doses of UTX-51 caused destructive dynamic damage against Gc protein during thermal neutron irradiation. These results suggested that borontrace drugs could be used as dynamic drugs for NDT targeted Gc protein for serum protein-quality-control treatment.

Y. Kinashi, K. Ono and S.Takahashi

Research Reactor Institute, Kyoto University

INTRODUCTION: We have described the mutation frequency of CHO cells after neutron irradiation and presented evidence for the increased mutagenicity of thermal neutrons [1,2]. High dose rate effect has been generally known in gamma or x-ray irradiation. On the other hand, little is known about dose rate effect of neutron irradiation. 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. 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 10B-para-boronophenylamine (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, to determine mutation frequencies, each treated culture was incubated with non-selective medium for 7-9 days to allow phenotype expression. Then, 2×10^5 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 with 10ppm BPA at 0.2Gy/min, without BPA at 0.2Gy/min or at

0.04Gy/min. The frequency of mutations after neutron irradiation with 10ppm BPA at 0.2Gy/min was two times and more increased than that without BPA 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.

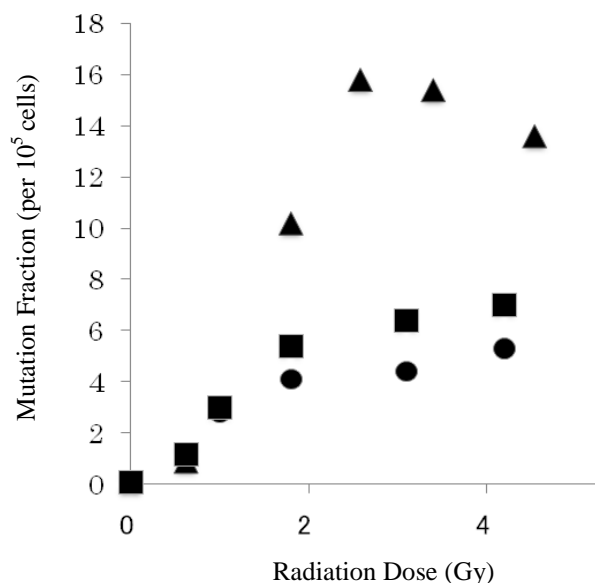


Fig.1 Induction of mutations in the HPRT locus in CHO cells after the neutron irradiation.

Plots of the mutant frequencies obtained with irradiated samples following KUR neutron irradiation with 10ppm BPA at 0.2 Gy/min (▲), without BPA at 0.2 Gy/min (■) and 0.04Gy/min (●) are shown. Plot of the mutant fraction as a function of the radiation dose.

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 ^7Li nuclei produced by $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction, not due to the reaction with normal tissue hydrogen and nitrogen.

REFERENCES

- [1] Kinashi Y *et al.*, Radiat Res., **154**(2000)313-318.
- [2] Kinashi Y *et al.*, Int J Radiat Oncol Biol Physics, **68**(2007)508-514.

PR8-2 Analysis of DNA Double Strand Breaks in Mammalian Cells Induced by BNCR

S. Takahashi, K. Okumura¹, Y. Kinashi, K. Ono, Y. Kubota² and R. Okayasu²

Research Reactor Institute of Kyoto University,

¹Graduate School of Agriculture, Kyoto University,

²National Institute of Radiological Sciences,

International Research Laboratory, National Institute of Radiological Sciences

INTRODUCTION: Boron neutron capture therapy (BNCT) is unique and effective treatment for cancer, and now becoming a clinical application stage. However, little is known about the biological effects of such radiation exposures as used 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. In the FY 2012, the induction of DNA double strand breaks (DNA-dsb) was detected by using the focus assay of repair protein.

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.

The radiation beam used was a mixed neutron beam consisting of the thermal, epithermal and fast neutrons, and gamma-rays. The detail of the irradiation conditions were described the 2010 Progress Report. 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.

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: The cell survival was determined by colony formation in both CHO/K1 and xrs-5 cells, respectively. As expected, xrs5 cells defective in repair were more sensitive to both mixed beam and gamma-rays irradiation than CHO-K1 cells.

The focus assay used for detecting the DNA double strand breaks showed that the number of gamma-H2AX and 53BP1 foci 1hr post-irradiation were similar for the mixed

radiation and the reference gamma-ray. 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 the mixed neutron beam and the reference gamma-rays.

The size of foci were also analyzed in some samples. As shown in Fig. 1, the mean of the foci sizes was not significantly different between the cell irradiated with gamma-rays and neutron, but the size distribution seemed to be larger in those irradiated with neutron.

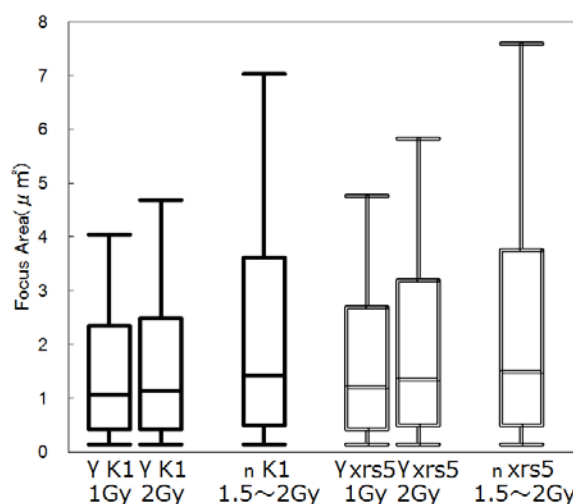


Fig. 1 Size distribution of foci in CHO (K1) and xrs cells after irradiation with gamma-ray and neutron beam. Larger foci are observed in the cells irradiated with neutron.

PUBLICATION:

K. Okumura, Y. Kinashi, Y. Kubota, E. Kitajima, R.Okayasu, K. Ono, and S. Takahashi, Relative biological effects of neutron mixed-beam irradiation for boron neutron capture therapy on cell survival and DNA double-strand breaks in cultured mammalian cells. J Radiat Res (2013) 54 (1): 70-75.

T. Morikawa, S. Iwamoto, Y. Hattori and
M. Kirihata

Graduate School of Life and Environmental Sciences, Osaka Prefecture University

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, gamma-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 detect the BNCR condition for leaf tissue culture and callus conditions. To determine the effectiveness of BNCR for plant mutagenesis at irradiation of dry seed, leaf disc and callus stages, haploid and diploid tobacco (*Nicotianatabacum L.cv. Red Russian*) plants were used because of its high callus induction, regeneration and totipotency.

EXPERIMENTS: The dry seed, leaf disc and callus of haploid and diploid tobacco plants were immersed in different concentrations (0, 400, 600, and 800 μ M) of ^{10}B -enriched *p*-boronophenylalanine (BPA) for 24hours, and all the materials were irradiated with thermal neutron for 0, 30 and 60 minutes in the Kyoto University Research Reactor (KUR). The irradiated seeds were kept at room temperature for overnight, and then were germinated on a plastic petri-dish containing moisture filter paper at 25 . The irradiated leaf disc and callus were instantly transferred to new MS medium with containing Auxin(2,4-D 2ppm) and Cytokinin(Kinetine 0.25ppm) for callus induction of leaf disc and callus sub-cultures. After regeneration of them the plantlets were transplanted to the pots filled with mixtures of soil and compost. Survival, dedifferentiation and regeneration rates were examined in the leaf discs and calluses.

RESULTS: The irradiated tobacco seeds were germinated normally at all fifteen treated groups (five BPA concentrations \times three irradiation

times) at the range of from 60% to 100%. There was no relationship between plant size of seedlings and dose effect of BPA and irradiation times. Tobacco seeds seem to be highly resistant to BNCR treatment. Survival, dedifferentiation and regeneration rates were also not affected with BPA concentrations in the leaf discs. Some mutants appeared in the M_1V_1 regenerated plants obtained from leaf disc calluses. For example, slender, dwarf, flower abnormal, male sterility, multi-branching and variegation plants were obtained in M_2V_1 generation. All irradiated calluses, however, were suddenly dead showing chlorosis during one month culture. This result indicated that tobacco callus is quite sensitive to BNCR because there is little protection of nuclear proteins during active somatic cell division and having weak single strand of DNA. There was no difference of BNCR sensitivity between haploid and diploid tobacco plants.

We have found out the optimum BNCR conditions in tobacco leaf discs, which were 400, 600 or 800 μ M BPA and 30 or 60 minutes irradiations for expecting the maximum mutation rate. This result indicates that the leaf disc of tobacco plants seem not to absorb enough amounts of BPA into the cells for overnight treatment.

H. Hori, R. Tada, Y. Uto, S. Takahashi¹ and K. Ono¹

*Institute of Technology and Science, Graduate School
The University of Tokushima*

¹*Research Reactor Institute, Kyoto University*

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).

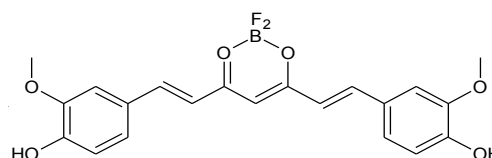
We previously developed boron tracedrugs UTX-42, UTX-43, and UTX-44, which possess antioxidant potency [1] and UTX-42, UTX-44, UTX-47, UTX-50, and UTX-51 as boron tracedrugs for NDT targeted a model protein BSA [2] and UTX-51 for LDL [3].

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 human serum Gc protein, which is related with serum protein-quality control, 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 serum Gc protein treated with the boron tracedrug UTX-51.

RESULTS: The combination of 0.5 nmol Gc protein (54 KD, 27 μ g) with three different B-10 concentration of 10, 25, and 50 nmol of the boron tracedrug UTX-51 (MW 416.12 g/mol; 50 nmol or 20.8 μ g, 125 nmol or 52.0 μ g, and 250 nmol or 104.0 μ g, for 10 nmol B-10, 25 nmol B-10, and 50 nmol B-10, respectively) showed a decrease in band intensity after neutron irradiation.

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



UTX-51

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

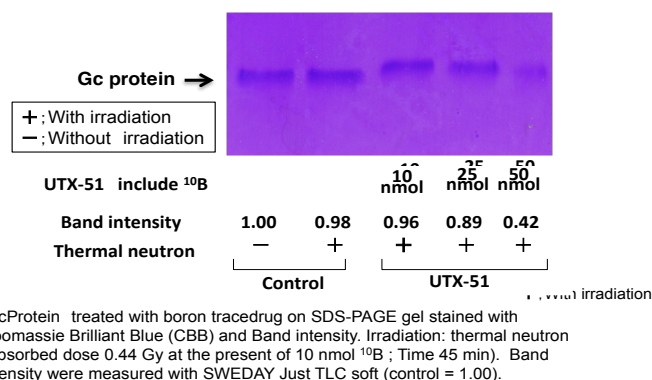


Fig. 2. NDT of Gc protein using a boron tracedrug UTX-51

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- [3] H. Hori *et al.*, *Adv. Exp. Med. Biol.*, accepted, 2013.