VIII-II-1. Project Research

Project 13

PR13 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. Since the Research Reactor (KUR) was not operated this fiscal year, setting up of equipments for experiments completed, and now it is ready for neutron exposure experiments.

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 Application of BNCR to plant seeds for mutation breeding
(K. Mishiba *et al.*)

PRS-5 Development of pharmacokinetic using boron trace drugs
(H. Hori *et al.*)

Main Results and Contents

PRS-1 and PRS-2 evaluated H2AX focus observed in the Ku80 deficient CHO mutant (xrs5) cells following gamma ray irradiation. Xrs5 (Ku80 deficient CHO mutant cells) are well-known for the DNA double strand breaks (DSBs) repair deficiency. The amount of DNA-DSBs following gamma irradiation in the xrs5 cells was evaluated phosphorylated H2AX (γ-H2AX) and 53BP1 foci using the immune-fluorescence intensity. Using the Boron Neutron Capture Reaction (BNCR), the ratio of non-reparable strand break / lethal event will be predicted to increase.

PRS-3 studied the application of BNCR to plant tissue culture. The aim of thePRS-3 study is to establish a novel mutagenesis system using BNCR in higher plants. To this end, the present work reports on the effect of ^{10}B-enriched *p*-boronophenylalanine (BPA) on regeneration of chrysanthemum (*Chrysanthemum morifolium* Ramat.) leaf tissues to gain fundamental knowledge of BPA uptake in plants. After 40 days of culture, reduction of regeneration frequency in the BPA-treated culture was observed; 36.7 % of the explants showed regenerated shoots in 400 μM BPA treated culture, whereas 71.7 % of the explants regenerated in the culture without BPA. To confirm the incorporation of BPA into the explant tissues during the culture, boron concentration in the cultured tissues was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). A 5.5-fold increase of boron concentration was observed in the tissues treated with 400 μM BPA compared to the control tissues, demonstrating that the BPA contained in the culture media was successfully incorporated into the explant tissues.

PRS-4 is planning a project to develop a novel plant mutation breeding method based on BNCR. To induce genetic mutation, BNCR needs to be carried out within nuclei with sufficient dose of alpha ray to induce DNA damage. The outline of PRS-3 is as follows: 1) appropriate condition for incorporation of ^{10}B-enriched *p*-boronophenylalanine (BPA) into plant seeds will be developed; 2) BPA-incorporated seeds will be subjected to different doses of thermal neutron irradiation by KUR; 3) phenotypic and genotypic changes in the seedlings exposed to thermal neutron will be analyzed to verify the effectiveness of the mutagenesis system. To this end, they first attempted to observe the effect of BPA application on germination of plant seeds.

PRS-5 designed, as traceable next-generation drug model, wholly innovative drugs named “boron trace drug”. They studied syntheses, physical properties, and pharmacological activities of newly designed phenolic BODIPY-containing antioxidants, such as UTX-42, UTX-43 and UTX-44, their architecture of which were basically composed from phenolic antioxidant moiety and stable boron-containing BODIPY moiety. Their inhibitory activities on mitochondrial lipid peroxidation were also investigated. Their further pharmacokinetic studies will be investigated in details for our purpose of development of pharmacokinetic methodology.

PR13-1 **Evaluation of H2AX Focus Observed in the Ku80 Deficient CHO Mutant (xrs5) Cells Following Gamma Ray Irradiation**

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INTRODUCTION: Xrs5 (Ku80 deficient CHO mutant cells) are well-known for the DNA double strand breaks (DSBs) repair deficiency. The amount of DNA-DSBs following gamma irradiation in the xrs5 cells was evaluated phosphorylated H2AX (γ -H2AX) and 53BP1 foci using the immune-fluorescence intensity.

METHODS: *Cell Irradiation/* CHO-K1 and xrs5 cells exponentially growing in MEM were trypsinized. The cell suspensions at 1 x 10^6 cells per 1.5ml eppendorf tube were irradiated at room temperature with Co-60 gamma-ray irradiation system at Research Reactor Institute of Kyoto University. *Immune-fluorescence staining of γ-H2AX and 53BP1/* Following irradiation, the cells were incubated at 37℃for times between 6-72 hours. Then, they were fixed in 3% formaldehyde/PBS. After the washing with PBS, cells were incubated with anti-phospho-Histone H2AX monoclonal antibody, Alexa Fluor 594, 53BP1 rabbit polyclonal antibody and Alexa Fluor 488. The localized foci of γ-H2AX and 53BP1 were counted under a Keyence fluorescence microscope (BZ-9000) and the intensity of the foci was analyzed by dynamic-cell-count system.

RESULTS and DISCUSSION: Figure 1, 2 shows the intensity of the γ -H2AX and 53 BP1 foci 6-72 hours post gamma-irradiation with 2Gy of 1Gy/min. The DNA-DSBs in the CHO-K1 cells were repaired after 24-76 hours following the irradiation. On the other hands, the amount of DNA-DSBs in the xrs5 was found to increase after 24-76 hours following the irradiation. 72 hours after irradiation, the number of γ -H2AX foci per xrs5 and CHO-K1 cell was estimated 20 ± 3 and 3 ± 1 respectively. The number of 53BP1 foci per xrs5 and CHO-K1 cell was estimated 7 ± 3 and 0.7 ± 0.5 respectively. Our results suggested that the most of the DNA-DSBs in xrs5 cells were not repaired 72 hours after 2Gy gamma-irradiation.

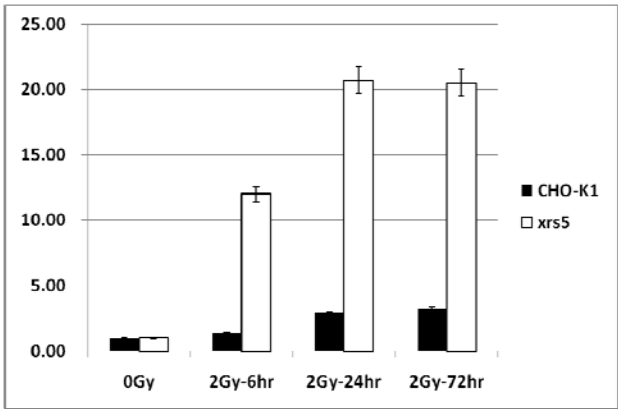

Fig. 1. The intensity of the γ -H2AX foci 6-72 hours post irradiation with 2Gy of 1Gy/min. gamma-rays. / □;xrs5 cells, ■; CHO-K1 cells.

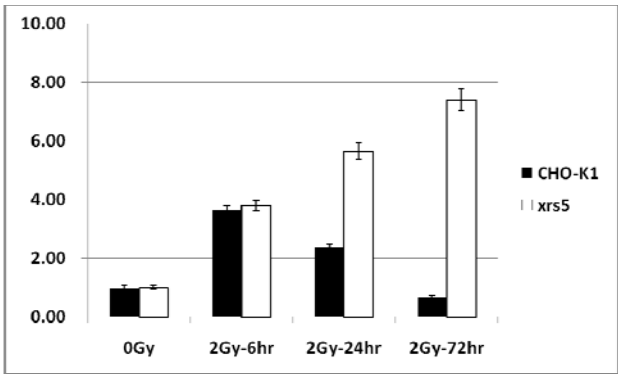

Fig. 2. The intensity of the53 BP1 foci 6-72 hours post irradiation with 2Gy of 1Gy/min. gamma-rays. /□;xrs5 cells, ■; CHO-K1 cells.

For comparison, cell survival was recorded using the colony forming assay. The parameter of D_{10} dose of CHO-K1 and xrs5 was 4.7Gy and 2.5Gy, respectively. For the two cell lines, the ratio of non-reparable DSBs of xrs5 : CHO-K1 is about 7-10 : 1, and the ratio of lethal event of xrs5 : CHO-K1 is about 2 : 1.
Using the Boron Neutron Capture Reaction (BNCR), the ratio of non-reparable strand break / lethal event will be predicted to increase. The study concerning the repair process of DSBs by BNCR will contribute to investigate the characteristic of the radiobiological effect of BNCT for malignant patients.

採択課題番号 21P13-1 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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PR13-2

Development of Pharmacokinetic Methodology Using Designed Boron Tracedrugs

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INTRODUCTION: The evaluation of ADME-tox and pharmacokinetic properties of drug candidates or even commercially available drugs as controls is extremely important and essential in the drug discovery process from early preclinical phase to phase IV, and more recently, in the advanced Ai (autopsy imaging) society. Recent increased needs of above evaluation accelerate medicinal chemistry researchers to develop drug candidates or new drugs with their own superior traceability. Traditionally, as well known traceable drugs, their radiolabeled compounds have been studied for their purposes. However, there are some inherent problems such as their half-life and regulation of experimental facilities. For the purpose of overcoming these problems and creating drugs with functions required for systems biology or emerging physiology, we designed, as traceable next-generation drug model, wholly innovative drugs named "boron tracedrug."

EXPERIMENTS: Our designed "boron tracedrugs" have their architecture of which were embedded boron atom in their scaffold or skeleton. If its boron-10, a naturally occurring and stable isotope (19.9%), produces prompt γ-ray after thermal neutron irradiation, the boron tracedrug located at the appropriate sites in the body could be detected easily. Utilizing this specific property, we expect that boron tracedrugs could show high traceability whenever they were required to be detected. As boron tracedrug c andidates, phenolic BODIPY-containing antioxidants, such as UTX-42, UTX-43 and UTX-44, as shown in Fig. 1, are sekected based on our molecular orbital approach in this study. UTX-42, UTX-43 and UTX-44 are synthesized from 1,3-dimetyl-BODIPY and their corresponding benzaldehydes via the Knoevenagel condensation

RESULTS: We studied syntheses, physical properties, and pharmacological activities of newly designed phenolic BODIPY-containing antioxidants, such as UTX-42, UTX-43 and UTX-44, their architecture of which were basically composed from phenolic antioxidant moiety and stable boron-containing BODIPY moiety. UTX-42, UTX-43 and UTX-44 were synthesized from 1,3-dimetyl-BODIPY and their corresponding benzaldehydes via the Knoevenagel condensation at their satisfactory yield. Their antioxidant activities were measured by spectroscopic analysis with ABTS: UTX-42 (IC_{50} = 6.8 μM), UTX-43 (9.5 μM) and UTX-44 (5.4 μM). Their inhibitory activities on mitochondrial lipid peroxidation were also investigated. Their further pharmacokinetic studies will be investigated in details for our purpose of development of pharmacokinetic methodology.

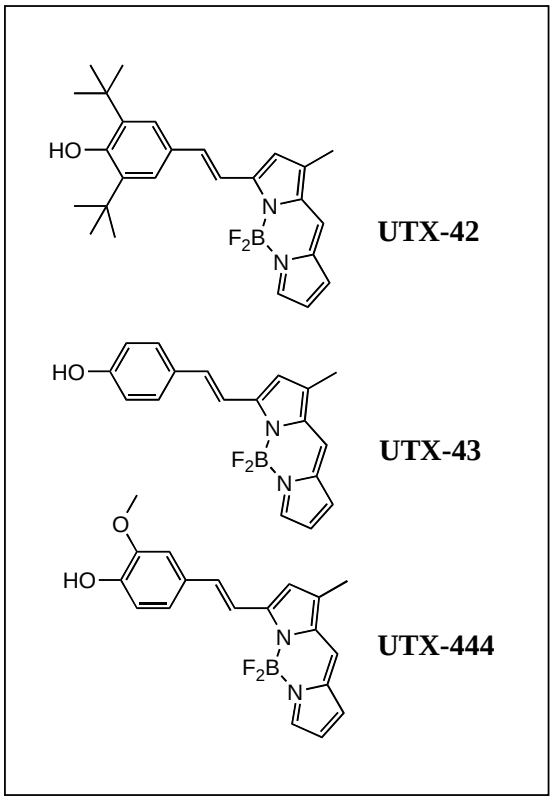

Fig. 1. Chemical Structures of designed "boron tracedrugs, UTX-42, UTX-43, and UTX-44.

採択課題番号 21P13-4　　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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