I. Project Research

Project 5

PR5 Project Research on a Study on Biological Character and Use of the Particle Induced by the Boron 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 par-ticles from the neutron capture reaction. Our project researchers were not able to carry out own experi-ments enough because KUR has been almost stop-ping during this year.

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 Development of the PARP repressor reinforced in its function by BNCR
(Y.Uto *et al.*)

PRS-4 Development of the model animal showing the blood vessel damage by BNCR
(R. Wate *et al.*)

Main Results and Contents

PRS-1 investigated that the biological effects of the combination of the BNCR (Boron Neutron Capture Reaction) and Temozolomide (TMZ) that is DNA alkylating agent on the T98G and A172 human glioblastoma cells were investigated. Our results suggested that TMZ treatment has no sensitization effect of radiation in A172 cells with low MGMT (methyl guanine methyl transferase) gene expression that is the DNA repair enzyme. T98G cells with high MGMT gene expression were resistance to TMZ. The enhancement effect of the combination of neutron irradiation and TMZ treatment was not found in T98G cells.

We were not able to carry out experiments sufficiently because KUR has been almost stopping during this year.

PRS-2 was not able to carry out its experiments because KUR has been almost stopping during this year.

PRS-3 was not able to carry out its experiments because KUR has been almost stopping during this year. The nuclear reactor operated again, but I did not get experiment days at my convenience.

PRS-4 was not able to carry out its experiments because KUR has been almost stopping during this year.

PR5-1 Biological Effects of DNA Alkylating Agents on the Cell Lethal Effects of BNCR

Y. Kinashi, R. Akayama and S. Takahashi

Research Reactor Institute, Kyoto University

INTRODUCTION: Most BNCT (Boron Neutron Capture Therapy) patients have already received chemotherapy. Especially many brain tumor patients are taking temozolomide (TMZ) treatment. TMZ is a DNA-alkylating agents and particularly effective cancer drug for glioblastoma. In this study, we investigated the sensitivity of the glioblastoma cell lines of T98G (p53 mutated) and A172 (p53 wild) after exposure to neutron irradiation and TMZ.

MATERIALS & METHODS: The glioblastoma cell lines of T98G (p53 mutated) and A172 (p53 wild) are purchased from Riken BRC Cell Bank. TMZ solution prepared by the medium, and removal washing with phosphate buffered saline after 23 hours incubation. In the experiment exposed with TMZ, T98G cells showed TMZ resistance about 10 times higher than A172 cells. The confluent cells in a culture dish were transferred into a Teflon tube and irradiated with a thermal neutron beam in the Research Reactor of In-stitute for Integrated Radiation and Nuclear Science, Kyoto University. A portion of the irradiated cells was checked to determine the cell survival rate using the conventional colony formation assay immediately af-ter irradiation. The gamma-rays irradiation for cells was carried out using Co-60 gamma-ray facility. After irradiation, cells were seeded on a Petri dish and incu-bated for 14 days. The survival curve creates a Plating Efficiency of each treatment and control groups com-pared survival rate.

RESULTS and DISCUSSION: T98G cells are resistance to TMZ because they have MGMT (methylguanine methyltransferase) gene expression that is the DNA repair enzyme. The IC50 value of TMZ for each cell line was 443 μM for the T98G and 43 μM for A172, respectively. Table 1 shows the survival data observed differences in sensitivity of the between two cells. The cell killing effect of neutron for each brain tumor cell was higher than gamma rays.

Table1. RBE (Relative Biological Effectiveness) calculated from D_{10} dose*

	A172	A172 +B10 (20ppm)	T98G	T98G +B10 (20ppm)
D_{10}(Gy) of neutron	1.7	0.82	5.2	1.1
D_{10}(Gy) of γ-ray**	4.8		7.0	
RBE	2.8	5.9	1.3	6.4

* Each D_{10} value was obtained from a survival curve
** Co^{60} gamma-ray system

Furthermore, the RBE of T98G was higher than A172 under the BNCR. These results indicate that BNCT is suitable for treatment of radio-resistant brain tumors.

In the combination study of neutron and TMZ, there was no apparent change in the D10 value of T98G cells. D10 value decreased slightly after the gamma-ray and TMZ in A172 cells. These results show that TMZ treatment has the sensitization effect of radiation in A172 cells. The A172 cells that alkylation repair activity is low and cells have high susceptibility to TMZ. TMZ concluded that further enhance the cell-killing effect of radiation. It is generally known that patients with glioblastoma with MGMT gene silencing have the benefit from TMZ and survival benefit for the chemoradiotherapy[1]. Further studies will be needed to analyze whether synergy effect of the combination of BNCT and TMZ in various brain tumors showing low TMZ expression.

[1] M.E.Hegi *et al.*, N. Engl. J. Med., **352**(2005)997-1003.