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Biological Effects of MC-50 Cyclotron Proton Beam Irradiation

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1. Introduction

The biological effect of space radiation is a new field of radiation biology, because human beings will explore more into space in this century. The charged particles are the main resources of space radiation outside the atmosphere. Proton comprises about 85% of space radiation and the rest 10% is composed of α-particle, heavy particle, and electron [1]. Therefore, it is very important to study the biological effects and countermeasures of charged particles for the evaluation of health problems and radiation protection during space exploration [2]. Although the biological effects of charged particles are being studied extensively in some countries including USA, Japan and China [3], little or no studies have been reported in Korea. Therefore, we aimed to obtain the basic data on the biological effects of high energy proton beam and to investigate the possible application of MC-50 cyclotron (KIRAMS, Korea) proton beam as a model for space radiation research. In this study, we investigated the oxidative damage by proton beam irradiation in cell culture system and in mice.

2. Methods and Results

2.1 Proton Beam Irradiation

Proton beam (34.9 MeV) irradiation was performed using MC-50 cyclotron at Korea Institute of Radiological and Medical Sciences, Korea. Cultured mammalian cells (CHO and Balb/3T3 cells) were irradiated in the polystyrene T-shape culture flask (5cm × 5cm) with 1-6 Gy of proton beam at 0.509 Gy/nA. C57BL/6 mice were confined in the acryl cage to minimize their movement and irradiated with 1-4 Gy of proton beam at 0.65 Gy/nA. The beam current was 1 nA.

2.2 Effects on Plating Efficiency and HPRT Mutation in CHO Cells

Chinese Hamster Ovary (CHO) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum. Cells were irradiated with 0-6 Gy proton beam and the media was replaced with fresh media within 30 minutes after irradiation. The plating efficiency (PE) was determined 24 hours after irradiation, and HPRT mutation assay was performed 6 days after irradiation, as described previously [4].

The plating efficiency (PE) showed sigmoidal curve with steep decrease from 87.5% to 38.3% between 2-4 Gy and slow descent at doses lower than 2 Gy or higher than 4 Gy (Fig. 1A). The mutation in hypoxanthine phosphoribosyl transferase (HPRT) gene is a widely-used biomarker in radiation biology since the mutants can be easily selected using the drug 6-thioguanine [5]. In our study, CHO cells were assayed for the induction of HPRT gene mutation 6 days after proton beam irradiation. The mutation frequency was 3.6×10^{-6} in naïve CHO cells and increased according to the proton beam dose, reaching 97×10^{-6} at 6 Gy (Fig. 1B). These results showed that proton beam induces cellar damage leading to the decrease of cell survival and the induction of mutations.

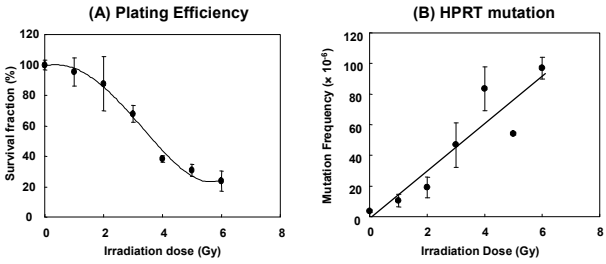

Figure 1. Effects of proton beam irradiation on the plating efficiency (A) and HPRT gene mutation (B) in CHO cells.

2.3 Effects on Stress Signaling in Balb/3T3 Cells

The cellular damage induced by irradiation activates the signaling pathways leading to the repair of damage, cell cycle arrest, or cell death [6]. To examine the effects of proton beam irradiation on stress signaling, the expression and phosphorylation of several signaling proteins in Balb/3T3 fibroblast cells were analyzed. Balb/3T3 mouse fibroblast cells were cultured in DMEM supplemented with 10% bovine calf serum. Cells were irradiated with 0-4 Gy proton beam and the media was replaced with fresh media within 30 minutes after irradiation. Cells were harvested at 2 and 4 hours after irradiation and protein level was analyzed by western blotting (Fig. 2).

The levels of IκB-α were decreased 2 and 4 hours after irradiation, which indicated the degradation of IκB-α and the subsequent activation of NF-κB. The protein level of p53 was increased at 4 hours after proton beam irradiation. The phosphorylation of p53 was evident at 2 and 4 hours after irradiation and showed dose dependency. Rb was slightly induced at 2 hours and 4 hours after irradiation. These results show that proton beam irradiation activates the DNA repair signal and alters the cell cycle progression.

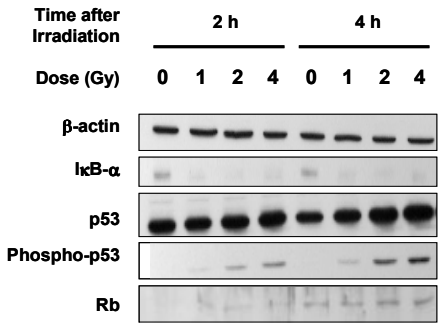

Figure 2. Effects of proton beam irradiation on the expression and activation of cell signaling proteins in Balb/3T3 cells.

2.4 Effects on Immune System in C57BL/6 Mice

It is reported that the immune system is greatly affected by space radiation and 1.0 Gy of solar proton can be lethal to the crew members [7]. Therefore, we investigated the effects of MC-50 cyclotron proton beam on the immune system in C57BL/6 mice. Female 10 weeks old C57BL/6 mice were irradiated with 0-4 Gy of proton beam. The number of immune cells (white blood cells and lymphocytes) in peripheral blood was counted before and 2, 8 days after irradiation (Fig. 3).

Two days after irradiation, the white blood cells and lymphocytes were reduced dose-dependently to 30-58% and 28-58% of the pretest values, respectively. These cells were partially recovered 8 days after irradiation, but the recovery was slower at higher doses of irradiation. These results suggest that 1-4 Gy of MC-50 cyclotron proton beam induces immune suppression in a dose-dependent manner, and the recovery from this suppression is slower in higher doses.

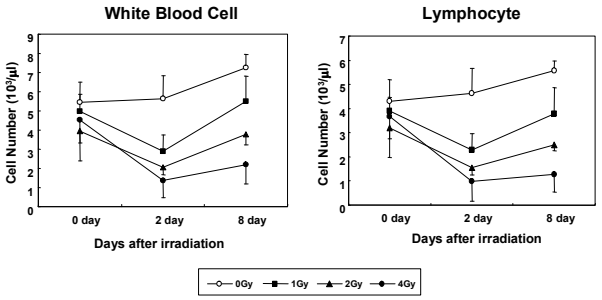

Figure 3. The white blood cell and lymphocyte numbers in peripheral blood of C57BL/6 mice after the proton beam irradiation.

3. Conclusion

In this study, we observed that the irradiation of proton beam generated by MC-50 cyclotron elicited several biological effects *in vitro* and *in vivo*. The plating efficiency reduction and HPRT gene mutation were observed in CHO cells. Several intracellular signaling pathways including NF-κB, p53 and Rb were activated in Balb/3T3 mouse fibroblast cells. Also the depression of immune system was observed in C57BL/6 mice. These results provided the basic data for further investigation on biological effects of proton beam. In addition, our results showed that MC-50 cyclotron proton beam induced the typical biological effects of space radiation and might be a useful model for space radiation research in Korea.

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