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EFFECT OF 90 MeV PROTON IRRADIATION ON SPLEEN INJURY IN C57BL/6J MICE

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Abstract

Proton therapy has become one of the most important physiotherapies for tumors in the world, which can greatly improve the cure rate of tumors that are ineffective by conventional treatments. In addition, proton is also the main source of radiation in space environment. Therefore, it is of great scientific significance to use accelerators to carry out basic research on proton radiotherapy and space radiobiology, which can provide technical support and basic data for the optimal design of proton therapy and risk assessment of personnel in space environment.

In this study, C57 mice were irradiated with 0, 0.2, 0.5 and 2Gy by 90 MeV protons from 100MeV cyclotron of China Institute of Atomic Energy. The mice were killed one day after irradiation. Body weight change and spleen organ coefficient were calculated. The expression of DNA damage-related protein γH2AX was detected by western blotting.

The results showed that compared with the control group, the body weight of mice in each irradiation group had no significant change, and the spleen organ coefficient decreased, indicating that the spleen atrophied after proton radiation, especially in 2Gy. The results of Western blotting showed that the expression of γ H2AX in spleen increased significantly on the 1 day after irradiation, especially in 0.5 and 2 Gy, indicating that the spleen DNA damage was the serious on the 1 day after proton radiation.

INTRODUCTION

With the development of China's aerospace industry, especially the advent of the space station era, the time spent by astronauts in space in the future will increase, and the impact of the space environment on the health of astronauts has also received more and more attention. Existing aerospace data show that medium and long-term space flight will affect multiple physiological systems of astronauts, such as genomic instability, cardiovascular function changes, bone loss, metabolic disorders, immune function changes and even the risk of carcinogenesis [1-3]. Space radiation is one of the important factors restricting manned spaceflight, protons are one of the main sources of space radiation, and its health effects on astronauts' health are particularly important [4].

The immune system is an important system for the body to perform immune responses and immune functions. It is composed of immune organs, immune cells and immune molecules. The immune system has the function of recognizing and eliminating antigenic foreign bodies, coordinating with other systems of the body, and jointly maintaining the stability of the environment and physiological balance in the body.

The spleen is the largest immune organ in the human body and the center of cellular and humoral immunity. Therefore, it is important to study the effect of proton radiation on the damage effect of the spleen.

In this study, 90 MeV medium energy protons generated by the 100 MeV proton cyclotron of the Chinese Institute of Atomic Energy were irradiated at different doses to mice, and the effect of 90 MeV proton irradiation on the spleen of C57 mice was studied. Spleen injury in C57 mice at different doses was obtained. In order to provide basic data and scientific basis for proton radiation protection.

MATERIALS AND METHODS

Animals and Experimental Design

Male C57BL/6J mice aged 6-8 weeks were obtained from SIPEIFU company, Beijing, China. The mice were acclimatized for a week under standard vivarium conditions. Next, we randomly divided the 24 animals into the experimental and control groups, including the control group of C57BL/6J(n=6), 0.2、0.5 and 2 Gy experimental group of C57BL/6J(n=6). At the end of the experiment, samples were collected on the first, third and seventh day.

Radiation Exposure

Proton irradiation was performed at the single-particle effect experimental terminal of CYCIAE-100 (Figs.1 and 2). Mice were exposed to 0.2、0.5 and 2 Gy of 90 MeV proton with 0.8 Gy/min. Control groups were subjected to a sham radiation procedure.

Figure1: The 100 MeV proton cyclotron.

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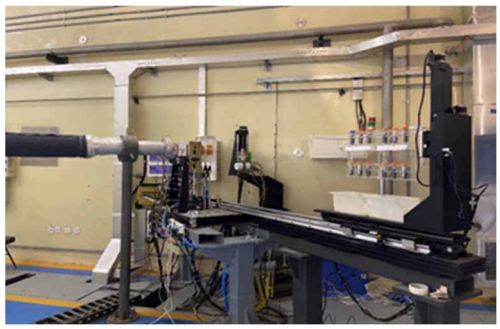

Figure2: The mice irradiation terminal diagram of cyclotron.

Weight and Organ Coefficients

All mice were weighed and recorded immediately after ear tags before irradiation, and weighed and recorded again before live killing, so as to obtain the weight changes of each group of mice before and after irradiation.

Remove the liver and place it on the filter paper, remove the residual water on the surface, weigh and record using an analytical balance, and the organ should correspond one-to-one with the mouse ear number. The organ coefficient is then calculated as follows:

Organ Coefficient (mg/g)= Organ Quality(mg)/ Weight(g).

Western Blot Experiment

Western blotting experiments were performed on mouse spleen 1 day after extraction. Tissue protein lysate was added to spleen and ground with a glass homogenizer. After centrifugation at 4℃, the supernatant was collected in EP tube and the protein concentration was measured by ultraviolet spectrophotometer. After protein denaturation-gel electrophoresis-film transfer-crosslinking-blocking, γ H2AX primary antibody was added and incubated at 4℃ (overnight), then incubated with corresponding secondary antibody at room temperature for 1 hour, and detected by chemiluminescence reagent kit. Finally, semi-quantitative analysis is carried out by Image J software.

RESULT AND DISCUSSION

The Results of Weight and Organ Index

The results showed that compared with the control group, the body weight of mice in each irradiation group had no significant change (Fig. 3). However, the spleen organ index of the irradiation group decreased significantly (p<0.05), indicating that after proton irradiation, the spleen atrophy of mice was most pronounced at 0.5 and 2 Gy (Fig. 4).

The Result of Western Blot

γ H2AX is a marker of DNA double-strand breaks. The results of western blotting showed that the expression of γ H2AX in the spleens of each irradiation group increased significantly 1 day after irradiation (p＜0.05), especially at 0.5 and 2 Gy (Fig.5), indicating that the DNA damage of the spleen was severe 1 day after proton irradiation.

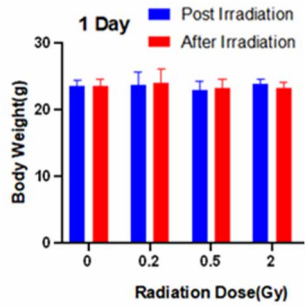

Figure 3: The effect of different doses of proton radiation on the weight change of mice.

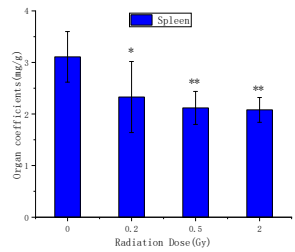

Figure 4: The effect of different doses of proton radiation on the changes in organ coefficients of spleen in C57BL/6J mice（vs 0 Gy,*p＜0.05，**p＜0.01).

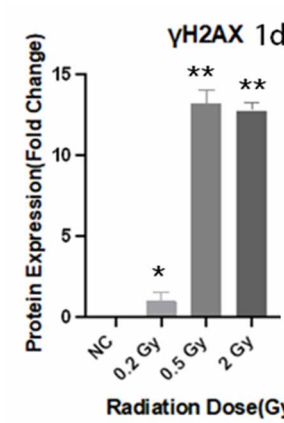

Figure 5: Semi-quantitative analysis results of γ-H2AX expression level in mice spleen tissue(vs 0 Gy,*p＜0.05，**p＜0.01).

Discussion

The organ coefficient, also known as the organ to body ratio, is generally a constant value, but when affected by external factors (such as ionizing radiation, drugs, etc.), some organs may be damaged, and their quality may change accordingly. Increased organ coefficient may be due to organ hyperemia, edema, or hyperplasia; Decreased organ factor may be due to organ atrophy or other degenerative changes. The results of this experiment showed that the spleen coefficient decreased significantly, indicating that the spleen is more sensitive to ionizing radiation, and proton radiation can cause spleen damage in the short term, destroy immune function, make the body in a state of susceptibility to infection, and is also an important cause of body death [5]. The organ coefficient is a very simple and

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clear indicator that can reflect the damage of proton radiation to the spleen of mice at the organ level.

In response to ionizing radiation-induced DNA DSB, H2AX is the earliest phosphorylated substrate, the most sensitive molecule in cells to sense DNA double-strand damage, can be used as a marker of DNA double-strand break damage, and the number of γH2AX foci and the number of DSBs are basically 1:1 in the number [6,7], which can achieve accurate quantification of DSB. In the results of western blot experiments on spleen DNA damage-related proteins at the molecular level, it is not difficult to find that protons can cause the expression level of DNA damage marker γ-H2AX to increase 1 day after irradiation, indicating that the more serious the DNA damage increases with the increase of proton irradiation dose.

CONCLUSION

In general, 90 MeV proton irradiation was used in C57 mice to study the effects of different doses of proton radiation on the spleen. The results showed that the spleen was highly sensitive to 90 MeV proton radiation, and atrophic degenerative changes began to occur at the 0.2 Gy dose. Further research on the DNA damage caused by proton radiation in spleen tissue found that 0.2 Gy proton radiation can induce spleen DNA double-strand breaks, and the DNA double-strand breaks become more severe with the increase of dose. It showed that proton radiation had a significant effect on spleen injury in mice, triggering the regulation of the immune system, thereby affecting the body's own damage repair.

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