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Low-dose carbon ion irradiation effects on DNA damage and oxidative stress in the mouse testis

Yang Liu [a,b,c,d], Jing Long [a,c,d], Luwei Zhang [a,c,d], Hong Zhang [a,c,d,*], Bin Liu [a,c,d], Weiping Zhao [a,c,d], Zhehua Wu [a,c,d]

[a] Department of Medical Physics, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, China
[c] Key Laboratory of Heavy Ion Radiation Medicine of Gansu Province, Lanzhou 730000, China
[d] Key Laboratory of Heavy Ion Radiation Biology and Medicine of Chinese Academy of Sciences, Lanzhou 730000, China

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Abstract

To investigate the effects of low-dose carbon ion irradiation on reproductive system of mice, the testes of outbred Kunming strain mice were whole-body irradiated with 0, 0.05, 0.1, 0.5 and 1 Gy, respectively. We measured DNA double-strand breaks (DNA DSBs) and oxidative stress parameters including malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, and testis weight and sperm count at 12 h, 21 d and 35 d after irradiation in mouse testis. At 12 h postirradiation, a significant increase in DNA DSB level but no pronounced alterations in MDA content or SOD activity were observed in 0.5 and 1 Gy groups compared with the control group. At 21 d postirradiation, there was a significant reduction in sperm count and distinct enhancements of DSB level and MDA content in 0.5 and 1 Gy groups in comparison with control. At 35 d postirradiation, the levels of DNA DSBs and MDA, and SOD activity returned to the baseline except for the MDA content in 1 Gy ($P < 0.05$), while extreme falls of sperm count were still observed in 0.5 ($P < 0.01$) and 1 Gy ($P < 0.01$) groups. For the 0.05 or 0.1 Gy group, no differences were found in DNA DSB level and MDA content between control and at 12 h, 21 d and 35 d after irradiation, indicating that lower doses of carbon ion irradiation have no significant influence on spermatogenesis processes. In this study, male germ cells irradiated with over 0.5 Gy of carbon ions are difficult to repair completely marked by the sperm count. Furthermore, these data suggest that the deleterious effects may be chronic or delayed in reproductive system after whole-body exposure to acute high-dose carbon ions.

Keywords: Carbon ions; DNA double-strand breaks (DNA DSBs); Malondialdehyde (MDA); Superoxide dismutase (SOD); Mouse testis

1. Introduction

Irradiation has been widely reported to damage organisms by attacking proteins, nucleic acid and lipids in cells.

Heavy ions, an important component of galactic cosmic rays, have high linear energy transfer (LET) and high relative biological effectiveness (RBE), and consequently are much more deleterious on the cellular or molecular level than low-LET irradiation such as X-rays or gamma(γ)-rays (Li et al., 2005). For example, heavy ions produce more irreparable DNA breaks (Ritter et al., 1997), chromosomal breakage and rearrangements and a greater degree of abnormal differentiation (Sekine et al., 2008) than low-LET irradiation. Due to the favorite characteristics of heavy-ion beams such as an energy deposition peak (Bragg peak) at the end of its range and an increased RBE within the peak, heavy-ion cancer therapy is attracting growing interest all over the world (Ji et al., 2008). However, heavy-ion irradiation not only destroys the tumor but could potentially damage the normal tissue around the tumor. In recent years, hormesis/adaptive response

* Corresponding author at: Department of Medical Physics, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China. Tel.: +86 931 4969171; fax: +86 931 8272100.
E-mail address: zhangh@impcas.ac.cn (H. Zhang).

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induced with low-dose ionizing irradiation to harmful effects of subsequent high-dose has become the focus of research in radiotherapy (Liu et al., 1987). Therefore, it is essential to monitor and evaluate the effects of heavy ion of low doses for human space exploration and heavy-ion cancer therapy.

The testis is one of the most radiosensitive organs in the body. There is abundant evidence from the animals that exposure of male germ cells to ionizing radiation cause detrimental influence (Hamer et al., 2003; Santra and Manna, 2009). Epidemiological data have suggested that the male germ damage induced by ionizing radiation may result in low reproductive ability and the presence of mutations due to pre-term pregnancy loss and various pathologies in offspring, including childhood cancer (Haines et al., 2002). Our previous findings also showed that heavy ions can lead to prominent morphological damage (Liu et al., 2009), and destroy poly (ADP-ribose) polymerase (PARP) activity and its expression linked with DNA repair (Zhang et al., 2008), and increase spermatocyte chromosomal aberrations (Zhang et al., 1998) in mouse testis.

Here, we investigated the dose- and time-response of low-dose heavy-ion irradiation on DNA double-strand breaks (DNA DSBs), oxidative stress index in mouse testes for better evaluation of the radiation risks in space and providing guidelines to protect gonad during radiotherapy.

2. Materials and methods

2.1. Animals

A total of 105 immature male Kunming strain mice (from Institute of Lanzhou Biological Products, China) weighing 16–18 g (about 3.5 week) were used in this study. All animals were housed in cages with free access to drinking water and diet. Cages were kept at 20–22 °C with 12 h light/dark cycle. Animals were randomly divided into six groups including control (0 Gy), 0.05, 0.1, 0.5 or 1 Gy group, each group constituted 21 male mice.

2.2. Irradiation procedure

Mice were restrained in containers fixed on the irradiation equipment at the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China). The carbon ion exposures were conducted at the therapy terminal of the HIRFL, which is equipped with a passive beam delivery system (Li et al., 2007). Animals were whole-body irradiated with carbon ion beam at 80 MeV/U and 31.3 keV/μm of the beam entrance, with a dose rate of 0.5 Gy/min. LET value was calculated using the HIBRAC code written by (Sihver et al. (1998). The collimation of the beams was controlled by a microcomputer. The acquisition of data (preset numbers converted by doses of irradiation) was automatically accomplished using a microcomputer during irradiation. Particle fluence was determined from the air ionization chamber signal according to the calibration of the detector (PTWUNIDOS; PTW-FREIBURG Co., Freiburg, Germany). All irradiations were performed at room temperature.

2.3. Evaluations of body weight, testis weight, sperm count

Seven of 21 mice from each group were used each time at 12 h, 21st day and 35th day after irradiation, respectively. The caudal epididymis and testis of each mouse were taken out. The fat and connective tissues adhering to testis were removed and the weight of two testes from each mouse was recorded. Then one testis was used for comet assay experiment and the other one was stored at −80 °C for the measurement of MDA level and SOD activity. The caudal epididymis were kept in 4 ml sodium chloride solution (0.86%) and minced finely by small scissors. The suspension was dispersed and filtered to exclude large tissue fragments. Sperm counts were recorded with the aid of hemocytometer under light microscope (Wyrobek et al., 1984).

2.4. Neutral comet assay

The methodology used in this study was similar to earlier studies (Quiles et al., 2004). Briefly, the cell suspension was mixed with 0.5% low-melting point agarose, which was kept in a 37 °C water bath. This suspension was rapidly removed from the first agarose and placed in 80 μL of 1% normal-melting point agarose that was spread on a frosted microscope slide and covered with a 24 × 24 mm coverslip. After gently removing the coverslip, the slide was then submersed in a lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10 and 1% Triton X-100 v/v, added just before use at 4 °C) for at least 1 h to lyse the cells. Next, slides were placed in an electrophoresis tank containing 0.3 M NaOH and 1 mM EDTA, pH 10, at 4 °C and incubated for 40 min to allow the separation of the two DNA strands (alkaline unwinding). Electrophoresis was performed at 1 V/cm and 300 mA for 30 min. Slides were then washed three times with a neutralizing solution (0.4 M Tris, pH 7.5) at 4 °C for 5 min per wash before staining with ethidium-bromide (5 μg/mL). The slides were covered with a coverslip and analyzed using a fluorescence microscope (Nikon). Images of 50 randomly selected nuclei per slide were analyzed using the Casp1.2.2 software (Institute of Theoretical Physics, University of Wroclaw). DNA DSBs were expressed as Olive tail moment (Konca et al., 2003).

2.5. Measurement of level of MDA and the activity of SOD

To evaluate oxidative status in the cells, the content of Malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) were measured in tissue homogenates of the testis using the commercial reagent kits (Jiancheng Bioengineering Ltd., Nanjing, China). In brief, frozen testis