Effects of low-dose heavy ion irradiation on male germ cell adaptation and genetics

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Abstract The heavy ions with high linear energy transfer and high relative biological effectiveness are much more deleterious on the male germ cells, ones of the most radiosensitive cells of the body, than low-LET ionizing radiation such as X-ray or gamma-ray. The effects of low-dose heavy ion irradiation on male germ cell adaptation and genetics and the possible mechanism of this adaptation are summarized in our laboratory. Our results showed that the heavy ion irradiation significantly increased the frequencies of chromosomal aberrations in spermatogonia and spermatocytes of mice, the low dose heavy ion irradiation could induce significant adaptative response on mouse testes and human sperm, and pre-exposure of mouse testes with low-dose heavy ion can markedly alleviate damage effects induced by subsequent high-dose irradiation. The increase of SOD activity and decrease of lipid peroxidation levels induced by low-dose ionizing radiation may be involved in this adaptative response mechanism. These studies may provide useful theoretical and clinical bases for radioprotection of reproductive potential and assessment of genetic risks for human exposed to heavy ions in radiotherapy and in outer space environment.

Key words Heavy ion irradiation, Low-dose pre-exposure, Male germ cells, Adaptative response, Genetics

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1 Introduction

Ionizing irradiation has been widely reported to damage organism by attacking proteins, nucleic acid and lipids in cells.[1,2] However, irradiation hormesis after low dose irradiation has been becoming the focus of research in radiobiology in recent years.[3,4] Many studies have shown that low dose ionizing irradiation can produce stimulating effects on the immune systems and induce adaptative response to harmful effects of subsequent high-dose radiation exposure.[3,5-7] Furthermore, the hormesis is exhibited not only in the whole-body level but also extensively in organ level, even in molecular level.[5-7] Most of these studies were performed with X-ray or γ-ray in the somatic. Comparing to X-ray or γ-ray, the track of a heavy ion is complex: energy is not only deposited by the primary interaction but also by secondary electrons which may travel considerable distances from the core. The heavy ions with high linear energy transfer (LET) and high relative biological effectiveness (RBE) are also much more deleterious on the cellular or molecular level than low-LET ionizing radiation, such as X-ray or γ-ray. For example, heavy ion irradiation induces unrepairable breaks in DNA more readily than low-LET irradiation.[8] Moreover, the testis is one of the most radiosensitive organs of the body and is the critical organ in the radiotherapy of Hodgkin's disease and seminoma.[9,10] It has been repeatedly demonstrated that asthenospermia, hypospermia, teratospermia and decrease in testis weight were associated with ionizing radiation.[11,12] With the advent of new radiotherapy modalities, such as fast neutrons and heavy ions, there is a considerable improvement in the survival rate of cancer patients. Thus, protection for reproductive potential and heredity in the germ cells of these patients against radiation damage is important. In this review we will summarize the effects of low-dose heavy ion

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irradiation on male germ cell adaptation and genetics and the possible mechanism of this adaptation.

2 Effects of pre-exposure with low-dose oxygen ions on mouse testicular structure and endocrine functions induced by subsequent high-dose irradiation

The testis, owing to its particularities in structure and functions, is one of the most radiosensitive organs of the body and shows degenerative changes after exposure to ionizing radiation. In order to investigate whether pre-exposure of low dose heavy ion could alleviate harmful effects induced by a subsequent high-dose (therapeutic dose) irradiation, we[13,14] radiated mouse testes with 2 Gy of $^{16}O^{8+}$ ion directly or pre-radiated testis with 0.05 Gy of $^{16}O^{8+}$ ion and then irradiated with high dose of 2 Gy at 4 h after per-exposure. Mouse body weight, testis weight, testicular structures and serum testosterone concentration were measured at day 35 after irradiation. The results showed that 2 Gy of $^{16}O^{8+}$ ion irradiation significantly diminished mouse body weight, testis weight and serum testosterone concentration, and impaired testicular structures which mainly presented the reduction of tubule diameter and the decrease or loss of germ cells in various developing stages, especially spermatogenic elements (Fig.1(3)). And changes in Leydig's or Sertoli's cells were also apparent, such as nuclei pyknosis and cytoplasm scantness (Fig.1(3)). It is known that testosterone is the androgen produced by testicular interstitial cells-Leydig's cells, which play important roles in maintaining spermatogenesis, stimulating development of male reproductive organs and promoting the protein synthesis. Reduction of testicular testosterone production induced by high dose irradiation is due to the altering of endocrine function of Leydig's cells and other interstitial elements by affecting steroid biotransformations in these cells,[15] which further results in diminishing of body weight and testis weight. However, pre-exposure mouse testes with a low-dose of $^{16}O^{8+}$ ion significantly alleviated above mentioned damage on body weight, testis weight, testicular structures (Fig.1(4)) and endocrine functions induced by subsequent a high-dose (2 Gy) irradiation. Our results suggested that low dose heavy ion irradiation could induce significant adaptative response on mouse testes.

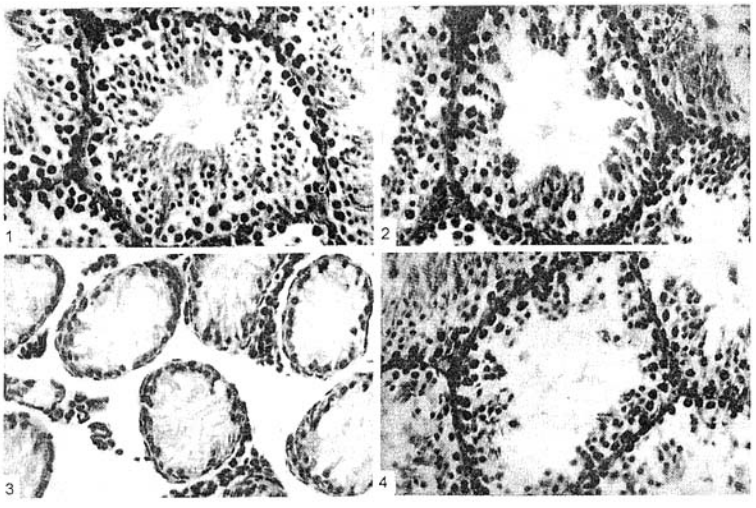

Fig.1 Testicular sections exposed to low-dose, high-dose and pre-exposure with low-dose and subsequent high-dose oxygen ion irradiation.[14]

(1) Control group. Tubule diameter, number and proportion of germ cells in various developing stages in the tubule and cells of the interstitial tissue were all normal. Hematoxylin-eosin stain, 250×. (2) 0.05Gy group. Tubule diameter, number and proportion of germ cells in various developing stages in the tubule and cells of the interstitial tissue were all normal. Hematoxylin-eosin stain, 250×. (3) 2Gy group. Tubule diameter markedly decreased, with reduction or disappearance of germ cells in various developing stages, nearly complete loss of the spermatogenic elements and the presence of significant number of Sertoli's cells. Most tubules were reduced to a single layer of epithelial cells. Leydig's or Sertoli's cells exhibited slight nuclei pyknosis and cytoplasm scantness. Hematoxylin-eosin stain, 250×. (4) 0.05Gy+2Gy group. Tubule diameter and the number of germ cells slight reduced. Leydig's and Sertoli's cells were morphologically normal. Hematoxylin-eosin stain, 250×.

3 Effects of pre-exposure of mouse testes with low-dose oxygen ions on mouse sperm count, shape abnormalities, superoxide dismutase (SOD) activity and lipid peroxidation induced by subsequent high-dose irradiation

To investigate the adaptative effects on male germ cells induced by low-dose heavy ion irradiation and the possible mechanism of this adaptation, we have conducted a series of experiments.[16] First we irradiated mouse testes with a low-dose of 0.05 Gy or a high-dose of 2 Gy $^{16}O^{8+}$ ion directly, or pre-exposed testes with 0.05 Gy of $^{16}O^{8+}$ ions and then, after 4 h, irradiated them with a challenge dose of 2 Gy. SOD activity and thiobarbituric acid reactive substances (TBARS) level (lipid peroxide level) in the testes were determined at 4 h after irradiation, and sperm count and sperm morphology were analyzed at day 35 after irradiation. The results showed that the SOD activity was enhanced by 10.3% and the TBARS level was decreased by 21.6% in the testes exposed to low-dose (0.05 Gy), and that sperm count was significantly reduced by 42% and sperm abnormalities were increased by 91% in the high-dose (2 Gy) group. Nevertheless, in the group of mice exposed to both the priming and challenge doses the damage effects on sperm count and sperm morphology were markedly alleviated, suggesting that pre-exposure of testis with a low-dose of heavy ions renders the organ more resistant to subsequent high-dose irradiation. The increase of SOD activity and the decrease of lipid peroxidation levels induced by low-dose ionizing irradiation may be involved in the mechanism of this resistance.[17] The process of adaptative response was thought to lead to the activation of protective and repair mechanisms of the body, including production of protective proteins and repair enzymes.[18] The adaptive responses of cells to low-dose ionizing irradiation or other agents were blocked by protein synthesis inhibitors.[19] The increase of radioresistance induced by low-dose irradiation may also be associated with free radicals which can also induce an adaptive response in low-dose. Pretreatment of Chinese hamster ovary or rat hepatoma cells with low non-toxic doses of H_2O_2 or xanthine-xanthine oxidase makes cells more resistant to the toxic and mutagenic effects of H_2O_2 and γ-ray.[20] Pretreatment of Chinese hamster V79 cells with low dose of H_2O_2 has also led to the observation of an adaptive response to a subsequent exposure of γ-rays or *N*–methy-*N*'-nitro-*N*-nitrosoguanidin.[21]

4 Effects of oxygen ion irradiation on human sperm spontaneous chemiluminescence (SCL), motility, acrosome reaction (AR) and viability in vitro

Sperm cell, owing to its specialization on structure and function, is known to be a radiosensitive cell in the body. To investigate the effects of heavy ion irradiation on human sperm and whether low doses of heavy ion irradiation could induce adaptative response in human sperm and possible mechanism, we have examined the human sperm SCL, motility, AR and viability induced by 0.25—64 Gy $^{16}O^{8+}$ ions irradiation.[21] The results showed that sperm SCL was significantly increased with irradiation doses and the lowest effective dose was 0.5 Gy. Motility of spermatozoa progressively elevated by 1, 1.2 and 1.3 fold with doses at 0.5, 1 and 2 Gy, respectively, and then significantly reduced with further increase of doses. The percentage of sperm AR markedly increased in 0.5—4 Gy and the optimal dose was 2 Gy with 2.7 fold enhancement, and then gradually decreased when irradiation dose was more than 4 Gy. Sperm viability had no significant change within 0.25—8 Gy, but was progressively decreased by 12.5%, 31% and 59% for 16, 32 and 64 Gy irradiation, respectively. Our results suggested that heavy ion at low doses increased sperm functions crucial to fertilization, for example motility and the percentage of AR, whereas heavy ions had deleterious effects at higher doses, which are probably associated with the bidirectional roles of free radicals produced by heavy ion irradiation.

5 Chromosomal aberrations induced by $^{12}C^{6+}$ ion or ^{60}Co γ-ray irradiation in spermatogonia and spermatocytes of mice

Chromosomal aberrations induced by irradiation in germ cells, which differ from those in somatic cells, not only indicate the cellular damage of radiated indi-

viduals, but also are partly transmitted to offspring and result in genetic effects, i.e. abnormalities, sterility and malignant diseases. Moreover, these radiotherapy patients of child-bearing age are concerned about risk of future children. Thus, it is important to ascertain whether the high LET radiation exposure increases the risk of chromosomal aberrations in gametes, so as to pay more attention to the reproductive potential and the possible genetic alteration in the germ cells of these patients.

The spermatogonia and spermatocytes are ones of the most radiosensitive cells of the body. Moreover, radiation-induced chromosomal aberrations of spermatogonia and spermatocytes were demonstrated to be transmitted into spermatozoa,[22] and these chromosomally abnormal sperm can also fertilize eggs. In order to investigate the effects of heavy ion exposure on the genetic risks of germ cells, we[23] analyzed the frequency and characteristic of chromosomal aberrations induced by 0.05—2.0 Gy $^{12}C^{6+}$ ion/^{60}Co γ-ray in spermatogonia and spermatocytes of mice. The results showed that there was an increase in frequency of chromosomal aberrations in all the treated groups compared to controls. There were 54.8% and 59.2% chromosomal aberrations for spermatogonia and spermatocytes exposed to 2 Gy of $^{12}C^{6+}$ ions, and 32.8% and 35.6% in the spermatogonia and spermatocytes which received 2 Gy of ^{60}Co γ-ray. The relative biological effectiveness (RBE) values of $^{12}C^{6+}$ ions with respect to ^{60}Co -ray were 1.67 for aberrations of spermatogonia and 1.66 for aberrations of spermatocytes for a dose of 2.0 Gy. Moreover, different distributions of various types of aberrations have been found for $^{12}C^{6+}$ ion and ^{60}Co γ-ray irradiations: in ^{60}Co γ-ray irradiation, the distribution was dominated by the chromatid break; while in $^{12}C^{6+}$ ion irradiation, the distribution of aberrations was clearly dominated by chromosome break and fragments. The dose–response relationships for $^{12}C^{6+}$ ion and ^{60}Co γ-ray exhibited that the slopes of the curves decrease with increasing in both spermatogonia and spermatocytes groups (Fig.2). Our results may provide useful information for assessment of genetic risks of human exposed to heavy ions. Radiosensitivity of germ cells is higher in human than in mouse. Hence there is a need for further investigation of the relationship of chromosomal aberrations induced by heavy ions between human and mouse germ cells, and finding out reasonable comparable rules among species.

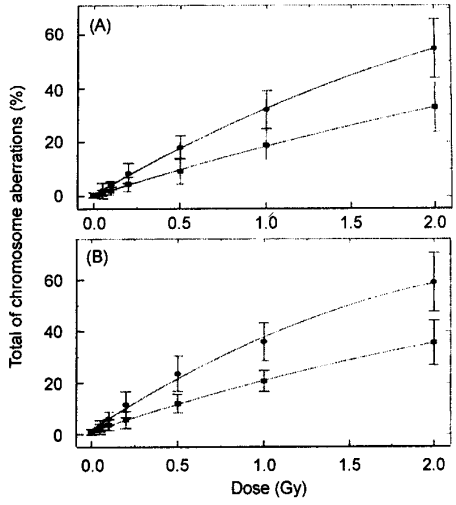

Fig.2 Dose–response relationships of chromosomal aberrations induced by $^{12}C^{6+}$ ion (●) and ^{60}Co γ-ray (■) in spermatogonia (A) and spermatocytes (B) of mice [16]. Data represent mean±SD, n=5.

6 Conclusion

In summary, the heavy ions with high linear energy transfer and high relative biological effectiveness are much more deleterious on the male germ cells, ones of the most radiosensitive cells of the body, than low-LET ionizing irradiation such as γ-ray. Pre-exposure mouse testis with low-dose heavy ions significantly alleviated the damages on testicular structure, endocrine, sperm amount and shape induced by subsequent high-dose irradiation. Increase of SOD activity and decrease of lipid peroxidation level induced by low-dose ionizing irradiation may be involved in this adaptative response mechanism. These results may provide useful theoretical and clinical bases for radioprotection of reproductive potential and assessment of genetic risks for human exposed to heavy ions in radiotherapy and in outer space environment.

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