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Evaluation of sex specificity on oxidative stress induced in lungs of mice irradiated by $^{12}C^{6+}$ ions

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Abstract The aim of this work is to identify if there is sex specificity on $^{12}C^{6+}$ ion-induced oxidative damage in mouse lung at different time points. Kun-Ming mice were divided into two groups, each composed of six males and six females: control group and irradiation group with a single acute dose of 4 Gy. Animals were sacrificed at 2, 4 and 12 h respectively, there lungs were removed immediately, and the oxidative stress-related biomarkers were measured by Diagnostic Reagent Kits. The results showed that the relative activities of superoxide dismutase (4 h), catalase (2 h) and Se-dependent glutathione peroxidase (12 h) have significant changes (P<0.05) between male groups and female groups, suggesting that the lungs of male mice are more sensitive to counteracting the oxidative challenge. Moreover, higher levels of malondiadehyde and lower contents of glutathione were also found in males, indicating that oxidative stress induced by $^{12}C^{6+}$ ion is pronounced in the lungs of males. We thought that these sex-responded differences may be attributed to the influence of sex hormones.

Key words Sex specificity, Oxidative stress, $^{12}C^{6+}$ ion, Mouse lung

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1 Introduction

In recent years, people are increasingly interested in the short wavelength free electron laser (FEL) when taking self-amplified spontaneous emission (SASE)[1] and high-gain harmonic generation (HGHG)[2-4] as two leading candidates for approaching hard X-ray region. However, presently the undulator period of FEL is in the order of centimeters[5-7], due to difficulties in placing strong and small magnets together into an alternating array. And because the FEL gain decreases rapidly with the undulator period, short-wavelength FEL must be achieved with high energy electron beam, which means enormous machines, substantive costs, and time and efforts consuming.

Heavy ion therapy is a promising technique for inveterate cancers because of its high linear energy transfer (LET) and high relative biological effectiveness (RBE), by producing DNA double strand breaks and irreparable DNA damage[1]. However, the tract of a heavy ion is more complex than other forms of ionizing radiation. For example, the primary interaction generates secondary electrons that may travel considerable distances[2]. It has been reported that heavy ions generate a variety of reactive oxygen species (ROS) including superoxid, hydroxyl radicals, singlet oxygen, and hydrogen peroxide[3-5]. Consequently, the ROSs leads to oxidative stress as a cause for cells death, aging processes, and mutagenesis. Therefore, it is important to study heavy ion-caused damages so as to prevent damaging the normal tissues and to obtain the best therapeutic effects.

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The lung is a relatively radiation sensitive organ, with complex response to irradiation, such as killing lung cells, death of endothelial cells and inflammatory cells, waves of inflammatory cytokines, and the occurrence of genomic instability as well[6]. As we know, some occurrences are associated with sex, such as morphological, physiological and pathological changes[7,8]. Silasi *et al* found that low-dose X-ray -induced effects were sex-specific and more pronounced in males[9]. Kovalchuk *et al* and Pogribny *et al* revealed that DNA methylation changes were more remarkable in tissue of male mice exposed to X-rays[10,11]. To the authors' knowledge, however, little has been known on whether sex specificity modulates heavy ion-induced oxidative damage. In this study, we evaluated the sex specificity on oxidative stress in lungs of mice irradiated by $^{12}C^{6+}$ ions.

2 Materials and methods

2.1 Animal

Approximately 3-week-old male and female Kun-Ming mice obtained from Lanzhou Medical College (Lanzhou, China) were used under identical breeding conditions. All animal studies were performed according to the requirements of the Animal Care Committee at the Institute.

2.2 Irradiation procedure

The mouse was positioned in a chamber fixed to a beamline at the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China) for whole body irradiation with $^{12}C^{6+}$ions of 80.55 MeV/u, which have an LET of 33.3 keV·μm^{-1} (in water) in the plateau region and 137.9 keV·μm^{-1} (in water) at the Bragg peak region. At a dose rate of 0.5 Gy·min^{-1}, a single dose of 4 Gy was delivered to the mouse. Under control of a microcomputer, the collimated beam scanned the animal until the preset ion number, which was converted from the dose, was achieved. The particle fluence was determined by an air ionizing chamber (PTW-UNIDOS, PTW-FREIBURG Co., Germany). The dose was calculated from particle fluence and LET.

2.3 Sample collections

Twenty-four Kun-Ming mice were divided into the control group and irradiation group. The control mice were sham treated. After irradiation, the animals (6 males and 6 females per group) were sacrificed at 2, 4 and 12 h respectively. The lungs were immediately frozen and stored at –80°C for biochemical determinations. The lungs of two animals were pooled in all the experiments.

2.4 Assays for enzyme activities

The samples were minced and homogenized in an ice-cold physiological saline solution. The supernatant fluid was obtained by centrifugation at 10 000 g for 30 min at 4°C. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH) and malondialdehyde (MDA) were assayed by Diagnostic Reagent Kits using colorimetric method (Nanjing Jiancheng Bioengineering, China) according to the manufacturer's instructions. Protein content was determined by the method of Bradford (1976), using bovine serum albumin (Sigma, USA) as standard.

2.5 Statistical analysis

Each experiment was repeated for at least four times. The results are expressed as means ± standard errors (S.E.M). The significance of differences between groups was determined by analysis of variance (ANOVA) with the multiple comparison tests. A P-value <0.05 was considered as a statistically significant difference. Correlation analyses were performed using SPSS 11.5 for WINDOWS.

3 Results

3.1 Changes of antioxidant status in lung induced by $^{12}C^{6+}$ ion irradiation

As shown in Fig.1, relative SOD activities of lungs of the irradiated male mice increased dramatically (P<0.01) at 4 h in comparison with the controls, while no notable change was observed in the female mice. In addition, there was a significant difference (P<0.05) between the female and male groups at 4 h.

In Fig.2, the relative activities of CAT of the

irradiated male mice were 2.2, 1.6 and 2.0 times higher than corresponding controls at 2, 4 and 12 h, respectively, while the relative activities of CAT of the irradiated female mice were 1.5, 1.1 and 1.4 times higher than corresponding controls at 2, 4 and 12 h, respectively. Moreover, a significant difference (P<0.05) was observed between female and male groups at 2 h.

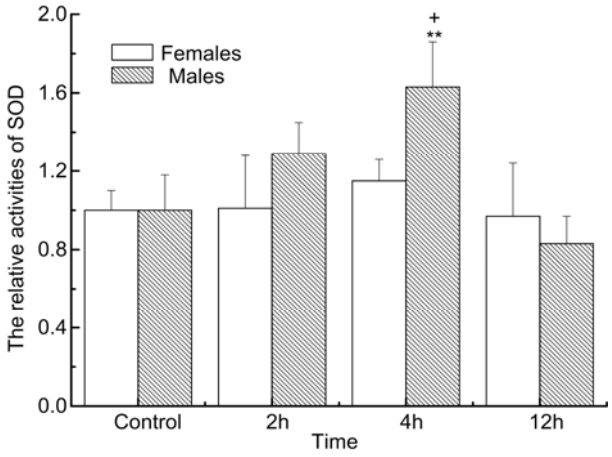

Fig.1 The effect of $^{12}C^{6+}$ ion on the relative activities of SOD in lungs between female and male mice. The female and male mice were treated with whole-body $^{12}C^{6+}$ ion irradiation (4Gy), and respectively sacrificed at 2, 4 and 12 h after exposure. Data represented means ± S.E.M (n=6). *P<0.05 for control groups *vs.* irradiation groups, and ^{+}P<0.05 for female groups vs. male groups.

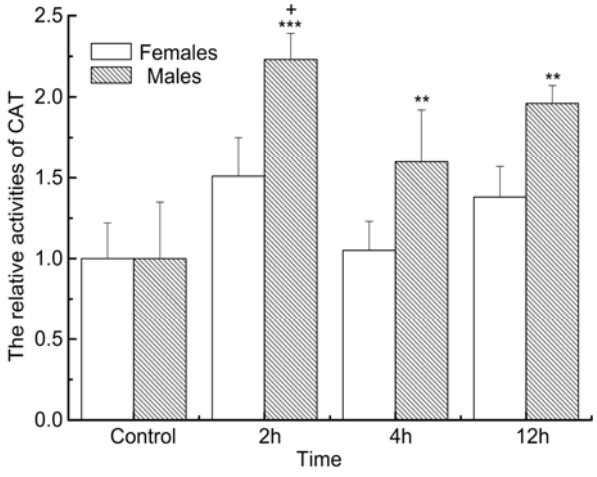

Fig.2 The effect of $^{12}C^{6+}$ ion on the relative activities of CAT in lungs between female and male mice. The female and male mice treated with whole-body $^{12}C^{6+}$ ion irradiation (4 Gy), and respectively sacrificed at 2, 4 and 12 h after irradiation.. Data represented means ± S.E.M (n=6). **P<0.01 and ***P<0.001 for control groups *vs.* irradiation groups, and ^{+}P<0.05 for female groups vs. male groups.

Compared to the control groups, there were no remarkable differences in relative changes of activities of GPx at all time points of the irradiated female mice (Fig.3). However, the relative activities of GPx of males decreased significantly at 12 h (P<0.01), and also a significant difference (P<0.05) was observed between female and male groups at 12 h.

Changes in GSH contents of the mouse lungs are shown in Fig.4. Relative GSH contents of the irradiated females reduced to 85%, 80% and 82% at 2, 4 and 12 h, respectively, while the relative GSH contents of males decreased to 64%, 69% and 62% at 2, 4 and 12 h, respectively.

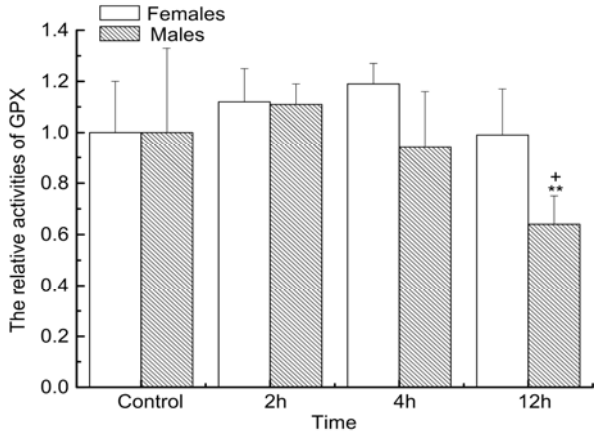

Fig.3 The effect of $^{12}C^{6+}$ ion on the relative activities of GPx in lungs between the female and male mice. Animals were treated with whole-body $^{12}C^{6+}$ ion irradiation (4 Gy), and sacrificed at 2, 4 and 12 h after irradiation, respectively. Data represented means ± S.E.M (n=6). *P<0.05 for control groups vs. irradiation groups, and ^{+}P<0.05 for female groups *vs.* male groups.

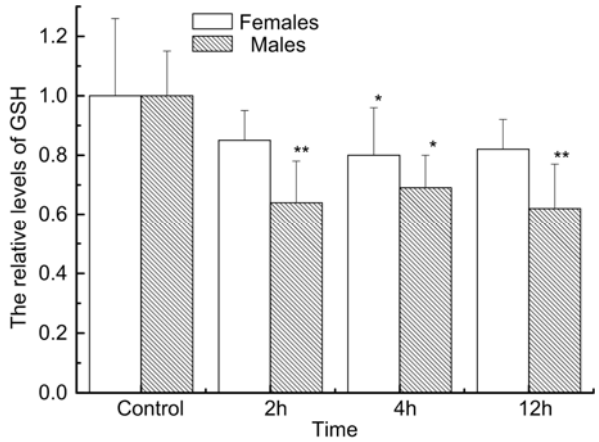

Fig.4 The effect of $^{12}C^{6+}$ ion on the relative contents of GSH in lungs between genders. The female and male mice were administered whole-body $^{12}C^{6+}$ ion irradiation (4 Gy), and respectively sacrificed at 2, 4 and 12 h after exposure. Data represented means ± S.E.M (n=6). *P<0.05, **P<0.01 for control groups *vs.* irradiation groups.

3.2 Changes of lipid peroxidation in mouse lung induced by $^{12}C^{6+}$ ion irradiation

Significant differences of lipid peroxidation (MDA formation) in lungs between the irradiated male and female mice were observed at 12 h (P<0.05) (Fig.5). For female mice, the ion-induced changes in relative levels of MDA were not significant (P>0.05) at any time point. However, the relative levels of MDA in the irradiated males were 1.2, 1.3 and 2.5 times higher than corresponding controls at 2, 4, and 12 h, respectively.

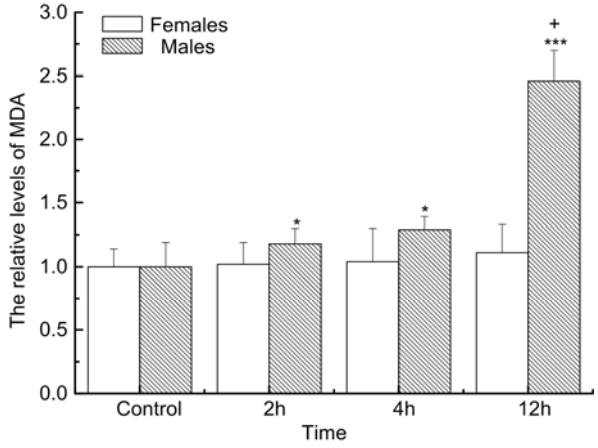

Fig.5 The effect of $^{12}C^{6+}$ ion on the relative levels of MDA in lungs between sexes. The female and male mice were administered whole-body $^{12}C^{6+}$ ion irradiation, and respectively sacrificed at 2, 4 and 12 h after irradiation. Data represented means ± S.E.M (n=6). *P<0.05 and ***P<0.001 vs. control groups for irradiation groups, and ^{+}P<0.05 for female groups vs. male groups.

4 Discussion

Ionizing radiations play an important rule in diagnosis and therapy, but they can damage organism by attacking proteins, nuclei acid and lipids in cells[12]. The initial interaction of ionizing radiations with a cell is the formation of ion pairs and electrically excited molecules[13]. Ion pair formation results in chemical changes and formation of reactive oxygen species (ROS), which are responsible for initiating biological damage, and further leading to cells death, aging processes, and mutagenesis[14,15].

Under normal conditions, cells have enzymatic and non-enzymatic mechanisms to scavenge ROS. Among the enzymatic defenses, the removal of damaging oxygen products is catalysed by SOD, Se-dependent catalase and GPx[16]. However, oxidative damage may occur when cellular antioxidant potential is changed and oxidative stress is increased, and consequently resulting in a number of injuries and diseases[17].

The MDA is a sensitive biomarker of oxidative stress[14]. Our results reveal that the increased levels of MDA induced by $^{12}C^{6+}$ ions in male lung were significant at 12 h, suggesting that a lot of ROS were accumulated in males after the irradiation. Furthermore, the sex-associated dissimilarities in oxidative stress may mainly be attributed to the influence from sex hormones. Estrogens were reportedly to have anti-inflammatory[18] and antioxidant properties[19], and even possibly mediated signaling in radiation response[7]. Moreover, higher gene expressions of antioxidant enzymes are found in females[20]. Additionally, the different responses to oxidative stress between genders might be relevant to difference of irradiation dose and metabolism.

SOD, CAT and GPx enzymes are crucial in the cellular defense against ROS. In response to $^{12}C^{6+}$ ion irradiation, the relative activities of SOD, CAT and GPx showed significant changes in males. These are similar to the results from Coto-Montes *et al* and Li *et al*[8,21]. The findings indicate that lungs of the males may be more sensitive to counteracting the oxidative challenge in acute lung injury. The occurrence of maximum activities of SOD, CAT and GPx at different time suggests that gene expression of antioxidant enzymes might be time-different in responding various ROS. However, this shall be further investigated.

GSH is the main intracellular antioxidant and has multiple biological functions. It acts directly as a scavenger of reactive oxygen species and as a substrate for GSH peroxidase to reduce hydrogen peroxide[22-24]. GSH protects against electrophiles and is also a vital determinant of cellular radiosensitivity because it can react with a variety of electrophilic compounds in the catalytic reaction of glutathione-S-transferase[25,26]. In this study, although the differences in GSH between female and male groups were not statistically significant, we found that the relative levels of GSH in the males were lower than the females, suggesting that irradiation-induced oxidative damage is more pronounced in male mice.

In conclusion, this study reveals remarkable

differences in oxidative-related parameters between lungs of male and female mice. The results manifest that there is a more serious oxidative stress in male mice after exposure to $^{12}C^{6+}$ ion. These sex-associated differences are thought to a possibility that estrogens most likely play a key role in regulating irradiation-induced oxidative damage.

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