Effect of N-acetylcysteine on $^{12}\text{C}^{6+}$ ion Irradiation-induced Lymphocytes DNA Damages and Immunity Changes in Mice

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$^{12}\text{C}^{6+}$ heavy ion/N-acetylcysteine/DNA double-strand breaks/Immunity acute effects.

The aims of present study are to estimate the biological risks to the immunity of mice exposed to heavy ion radiation and to investigate the effect of N-acetylcysteine (NAC) on $^{12}\text{C}^{6+}$ ion irradiation-induced lymphocyte DNA damage. Results showed that in the brine group, the levels of lymphocyte DNA damage and MN, thymocytes G$_2$/M phase arrest and apoptosis percentages (except for activity of NK cells) were up at each time point. A time-response curve for MN and DNA damage appeared in the NAC group. We found that whole-body $^{12}\text{C}^{6+}$ ion irradiation at a dose of 4 Gy could induce lymphocyte DNA double-strand breaks (DSBs); immunocytes DSBs may lead to acute effects on immunity; and 200 mg/kg NAC showed significant protection against radiation harm.

INTRODUCTION

One of the challenges of space exploration is the biological effects from exposure to heavy ion radiation levels so evaluation of potential health effects from radiation exposure is important for the astronaut and terrestrial humans who are continuously and simultaneously exposed to numerous environmental radiations. Recently, it became more and more important to determine the time-effect relationship of radiation exposure when assessing human health risks. Most of these studies have been performed with irradiation of high doses, and they had less relation to medical protective effect on immunity of body exposed to irradiation. Moreover, there has been growing attention paid to changes in the immune system after irradiation. Numerous studies have demonstrated that the immune competence of irradiated human populations, as well as experimental models in the laboratory declined with increasing radiation dose of various radiation types.$^{1-3}$ Changes in immune function are also reported during space flight.$^{4}$ Heavy ion radiation could lead to cellular DNA damages, which are the most important radiation injuries leading to cell death.$^{5}$ And such damages result in base substitutions, insertions and deletions, which might conduct to point mutations of DNA,$^{6}$ and these mutations play central roles in carcinogenesis.$^{7}$ In order to elucidate the influence of DNA strand breaks in immunocytes induced by heavy ion irradiation and NAC protection from radiation, we have examined the lymphocyte DNA and chromosomal damages, the changes of cell cycle and apoptosis of thymocytes, and the activity of natural killer (NK) cells. The data may provide new evidences on the mode of DNA strand breaks and an experimental base for radioprotection of immunity in human exposed to irradiation environment.

MATERIALS AND METHODS

Animals

Outbreed Kun-Ming mice (6~7 weeks) weighing 23 ± 1 g were provided by the Institute of Veterinarian in Lanzhou, the Chinese Academy of Agricultural Science. They were randomly divided into 3 groups: the control (4 animals) group, NAC-treated group and brine group with 16 animals of equal number of sexes in each group. NAC (200 mg/kg) dissolved in brine (0.85%) and the same volume of brine (0.85%) was given by celiac injection in 1 h before irradiation, respectively. The studies were approved by the Animal Care Committee at the Institute.

Irradiation

Each mouse was positioned in a chamber which was fixed...
to the irradiation equipment at the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Science, Lanzhou, China). The animal was whole-body irradiated individually with carbon ions, the energy of the ion beam was calculated to be 89.63 MeV/μ, corresponding to a linear energy transfer (LET) 28.3 keV/μm in the water and the dose rate was adjusted to be about 2 Gy/min. The dose used for this experiment was 4 Gy. This experiment was only completed once.

Measure

The relative thymus and spleen weights were measured after exposure 0.5, 2, 4 and 12 h,8,9 the peripheral blood lymphocytes DNA damages and chromosomal effects were quantified by the neutral comet assays10,11 and micronuclei (MN);12 the cycle and apoptosis of thymocytes were determined by flow cytomtery13 and the activity of splenic NK cells was measured by MTT.14

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\text{Thymus index} = \frac{\text{thymus weight (mg)}}{\text{body weight (g)}} \times 10
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\text{Spleen index} = \frac{\text{spleen weight (mg)}}{\text{body weight (g)}} \times 10
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Each value was expressed as the mean ± SD. An ANOVA analysis of variance was used to determine the level of any statistically significant differences between irradiated and unirradiated control groups. A p-level of 0.05 or less was selected as a criterion for a statistically significant difference.

RESULTS AND DISCUSSION

In this study, the brine group is more effective in drawing broken DNA away from the NAC-treated group in Fig. 1. Not only the tail appears longer, but more DNA is found within the tail (Fig. 1). The significant increases of OTM (except for 0.5 h), TDNA and TL (except for 0.5 h) appeared in each time point (Fig. 2). DNA is thought to be the critical target of biological effects of radiation by both direct energy deposition on DNA (direct effect) and reactions with diffusible water radicals (indirect effect).15 In the comet assay, the high number of damaged cells in mice indicated that the whole-body heavy ion irradiation on mice could induce lymphocyte DNA double-strand breaks (DSBs) primarily.16 DSBs are the most severe form of DNA damage as even a single strand break has the potential to induce the cell death. And as we know, X-ray irradiation with low-LET induced cellular single-strand break,17 but the heavy ion irradiation with high-LET has higher relative biological effect (RBE) than that of low-LET irradiation.18 Therefore, the DNA
damages induced by heavy ion may be more serious compared to damages from low-LET irradiation and more difficult to repair. In our experiments, the level of DNA fragmentation of lymphocytes has a significant augment in 2 h after irradiation, and there were about 236.84% of OTM, 90.95% of TDNA and 170.05% of TL increases compared to control (Fig. 2). The mechanisms underlying this phenomenon probably related to the irradiation damages and cellular repair capacity. Ion irradiation might cause DSBs of genomically unstable cells from 0.5 to 2 h after irradiation. The level of DSBs reduced with time to allow some genotoxic damages recovered, and cell population survival was as a priority. However, there is no evidence to support this hypothesis. Otherwise the competence of immune system recovered little in 12 h after radiation in brine group (Fig. 2). And genomic instability is characterized by a number of delayed responses including chromosomal abnormalities, gene mutations and cell death. In this study, the more DSBs might strengthen the lymphocytes chromosomal instability (Fig. 3), thymocytes G2/M phase arrested and apoptosis increases (Table 1), splenic NK cells activity decreases (Fig. 4) and thymus atrophy (Fig. 5).

In NAC-treated group, level of DSBs in 0.5 h was lower than control group. Time-response curve for DNA damage are shown in Fig. 2. This phenomenon could be related to the effect of NAC, a well-known antioxidant, glutathione precursor and containing one –SH group, which can raise the intracellular concentrations of GSH and scavenging ROS. In our study, NAC-treated mice have less lymphocytes DSBs, which might strengthen the level of NK cell activity (Fig. 4), and influence changes of peripheral blood lymphocytes MN (Fig. 3) and apoptosis of thymocytes (Table 1). And the effect of NAC became more and more weakly with body metabolism with time. More data at later time points after radiation exposure are needed. In our experiment, DSBs data could indicate that the mouse peripheral blood lymphocytes treated with NAC was less vulnerable to radiation-induced DNA fragmentation compared with those without NAC, which indirectly suggested that this difference in DSBs might be related to the ROS. The peripheral blood lymphocytes DSBs might be caused by ROS primarily which was induced by irradiation, and direct effect on DNA of heavy ion radiation in this experiment might be neglected. Then immunocyte DSBs might affect the immunity of body, and 200 mg/kg NAC showed a significant protection against radiation harm.

Table 1. Changes in the cell-cycle distribution and apoptosis of murine thymocytes at 0.5, 2, 4 and 12 h after exposure to carbon ions. (X ± S)

| Group   | G0-G1 (%) | S (%) | G2-M (%) | Apoptosis (%) |
|---------|-----------|-------|----------|---------------|
| Control | 68.61 ± 1.61 | 12.95 ± 1.45 | 18.42 ± 0.84 | 1.71 ± 0.59 |
| NAC (0.5) | 64.91 ± 1.34 | 1.87 ± 1.27 | 33.24 ± 3.6 | 12.94 ± 1.83 |
| Brine (0.5) | 60.75 ± 1.40 | 1.72 ± 1.25 | 37.48 ± 4.19 | 16.07 ± 2.55 |
| NAC (2) | 69.58 ± 1.65 | 5.12 ± 1.33 | 25.30 ± 1.51 | 12.14 ± 0.55 |
| Brine (2) | 70.30 ± 0.80 | 3.72 ± 0.5 | 26.23 ± 0.69 | 11.72 ± 1.03 |
| NAC (4) | 71.73 ± 0.94 | 7.03 ± 0.75 | 21.25 ± 1.56 | 8.88 ± 0.73 |
| Brine (4) | 70.16 ± 2.78 | 4.58 ± 0.95 | 25.26 ± 2.16 | 12.95 ± 1.76 |
| NAC (12) | 69.1 ± 1.44 | 5.99 ± 2.65 | 24.91 ± 3.82 | 9.91 ± 0.46 |
| Brine (12) | 63.48 ± 3.9 | 5.07 ± 1.29 | 31.56 ± 2.73 | 10.79 ± 1.40 |

*p < 0.05, **p < 0.01, ***p < 0.001 vs. control.
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