ChinaRxiv 中X

AI translation · View original & related papers at
chinarxiv.org/items/chinaxiv-202306.00186

N-acetylcysteine protects lymphocytes and cytokines against heavy ion irradiation via counteracting glutamate (Postprint)

Authors: XIE Yi, ZHANG Hong, WANG Yipei, LIU Yang, CHEN Yingtai

Date: 2023-06-18T00:00:00+00:00

Abstract

We evaluated the protective effect of N-acetylcysteine (NAC) on immunity system irradiated by 12C6+ ion beam. Kun-Ming mice were whole-body irradiated by 12C6+ ion at doses of 0, 0.5, 1, 1.5, 2, 2.5 and 3 Gy. The results showed that in saline group, the lymphocytes DNA double-strand breaks (DSBs), maleic dialdehyde, thymocytes number in G0/G1 and apoptosis percentage increased with dose increment, and the levels of interferon-γ, glutathione, superoxide radical (SOD) and natural killer cells activity decreased with dose increment. However, there were no significant changes in NAC-treated group. The data indicated that pre-treatment with NAC could significantly remove the ROS by counteracting the glutamate, decrease excessive lipid peroxidation reaction and SOD damages, and protect DNA, lymphocytes and cytokines against irradiation.

Full Text

N-acetylcysteine Protects Lymphocytes and Cytokines Against Heavy Ion Irradiation via Counteracting Glutamate

Yi Xie[123], Hong Zhang[123]*, Yipei Wang[4], Yang Liu[123], Yingtai Chen[5]

[1]Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, China
[2]Key Laboratory of Heavy Ion Radiation Biology and Medicine of Chinese Academy of Sciences, Lanzhou 730000, China
[3]Key Laboratory of Heavy Ion Radiation Medicine of Gansu Province, Lanzhou 730000, China
[4]Department of Chemistry & Biochemistry, University of Delaware, Newark,

DE 19711, USA
[5]Department of Abdominal Surgical Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing 100021, China

Abstract

We evaluated the protective effect of N-acetylcysteine (NAC) on the immune system of Kun-Ming mice whole-body irradiated with a 12C6+ beam at doses of 0, 0.5, 1, 1.5, 2, 2.5, and 3 Gy. In the saline-treated group, lymphocyte DNA double-strand breaks (DSBs), malondialdehyde levels, the percentage of thymocytes in G0/G1 phase, and apoptosis all increased with radiation dose, while interferon-γ levels, glutathione, superoxide dismutase (SOD) activity, and natural killer cell activity decreased with dose. However, no significant changes were observed in the NAC-treated group. These data indicate that pre-treatment with NAC could effectively scavenge reactive oxygen species (ROS) by counteracting glutamate, reduce excessive lipid peroxidation and SOD damage, and protect DNA, lymphocytes, and cytokines against irradiation injury.

Key words: N-acetylcysteine, 12C6+ heavy ion, DNA damage, oxidative stress, lymphocytes and cytokines

Introduction

Humans are constantly exposed to ionizing radiation from natural sources such as cosmic rays (during space travel) and radioisotopes in the earth's crust, as well as from numerous artificial sources. Currently, ionizing radiation is extensively applied in power generation, developing new high-yield crop varieties, sterilization, and extending food storage periods (food irradiation). With such widespread applications, human exposure has become inevitable. Although the main health risks during radiation exposure remain poorly understood, reports have documented risks of carcinogenesis and degenerative disease [1].

Cellular damage caused by ionizing radiation is predominantly mediated through free radicals and resultant reactive oxygen species (ROS) [2]. Primary radicals generated during water radiolysis react with molecules such as oxygen to produce secondary radicals (H2O2 and O2−), which can damage vital cellular targets including DNA, proteins, and membranes, ultimately leading to cell death and cancer [3]. Recently, many studies have focused on the potential use of thiols as free radical scavengers to prevent oxidative damage.

Glutathione (GSH) is the principal intracellular thiol responsible for scavenging ROS and maintaining oxidative balance in tissues. Cysteine and GSH delivery compounds have been used to protect normal cells from redox imbalance [4]. N-acetylcysteine (NAC) is one of the most widely used thiol antioxidants; it indirectly replenishes GSH through deacetylation to cysteine, thereby preventing oxidative damage via ROS scavenging [5].

The immune system is one of the most radiosensitive systems in mammalian

organisms. The thymus provides the essential microenvironment where T-cell precursors (thymocytes) mature in higher vertebrates. Natural killer (NK) cells function in innate immunity and are crucial for combating viral infections and destroying cancer cells [6,7]. Low NK cell activity is associated with increased cancer risk. Interferon-γ (IFN-γ) is a pleiotropic cytokine that plays important roles in regulating inflammatory and immune responses [8-10].

Based on these observations regarding radiation effects and thiol protective capabilities, we investigated the protective effects of NAC on the murine immune system against heavy ion irradiation and the possible underlying mechanisms.

2.1 Animals

Outbred Kun-Ming mice (6–7 weeks old) of both sexes, weighing 23 $\pm$ 1 g, were provided by the Institute of Veterinarian Agricultural Sciences in Lanzhou, Chinese Academy of Sciences. Animals were randomly divided into three groups (control, NAC-treated, and saline-treated), with 36 animals per group (6 per subgroup). One hour before irradiation, NAC (200 mg/kg) was dissolved in saline (0.85%) and administered to mice via intraperitoneal injection; the saline-treated group received the same volume of saline. This study was approved by the Animal Care Committee of the institute.

2.2 Irradiation

The carbon ion beam was provided by the Heavy Ion Research Facility in Lanzhou (HIRFL, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou, China). Animals were whole-body irradiated individually in the plateau region (energy: 284.7 MeV/u, linear energy transfer (LET): 25.6 keV/ m, dose rate: [preserved as in original]).

2.3 Levels of Superoxide Dismutase (SOD) Activity, Malondialdehyde (MDA), GSH, and IFN-γ in Serum

Blood samples were collected from the orbital sinus two hours after irradiation, after which mice were sacrificed by cervical dislocation. The thiobarbituric acid (TBA) method of Hodges [11] was used to determine lipid peroxidation by measuring TBA-reactive substances. Commercial detection kits for MDA were analyzed using an ultraviolet spectrophotometer (Nanjing Jiancheng, China). SOD activity was determined spectrophotometrically [12] using the -nitroblue tetrazolium chloride (NBT) reduction to formazan method. GSH levels were measured spectrophotometrically in the supernatant at 412 nm using 5,5 -dithiobis(2-nitrobenzoic acid) (412 = 13,600 M−1cm−1) [13]. IFN-γ levels were detected using commercially available enzyme-linked immunosorbent assay (ELISA) kits specific for mice (eBioscience, San Diego, California, USA).

2.4 Comet Assay of Splenic Lymphocytes

We used a modified neutral comet assay procedure to quantitatively measure DSBs in splenic lymphocytes [14]. Lymphocyte separation medium (LSM) (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd, Shanghai, China) was used for low-speed isolation centrifugation (300×g for 20 min). Digitized images were analyzed using professional Comet Assay Software Project (CASP, Wrocław, Poland).

2.5 Levels of NK Cell Activity in Spleen

NK cell activity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay [15].

2.6 Cell Cycle and Apoptosis of Thymocytes

Thymocyte cell cycle and apoptosis were measured by flow cytometry (Becton Dickinson). Cells were stained with propidium iodide (PI, Sigma-Aldrich, Missouri, USA) only [16]. Data were acquired using FACScan software (Becton Dickinson, New Jersey, USA) and analyzed using ModFit LT 3.0 software (Becton Dickinson).

2.7 Statistical Analysis

Values are expressed as mean ± SD. ANOVA was used to determine statistically significant differences among NAC, saline, and control groups. A p-level of 0.05 or less was selected as the criterion for statistical significance.

3 Results and Discussion

Heavy ion irradiation is well known to produce ROS [17]. In this study, the olive tail moment (OTM) of splenic lymphocytes increased with dose in the saline group, rising by 220% at 3 Gy compared to controls (Figs. 1a and 1b). Excessive DNA double-strand breaks (DSBs) indicate decreased cell activity and increased apoptosis. Our data showed a dose-dependent increase in DSBs in the saline group. Studies have reported that DSBs are primarily caused by ROS induced by irradiation [18]. The high levels of DNA damage indicated that heavy ion irradiation could induce overactive ROS accumulation, with ROS levels increasing with dose.

Increased ROS generation may trigger lipid peroxidation. MDA is an end product of lipid peroxidation, defined as oxidative deterioration of polyunsaturated lipids. As shown in Fig. 2a, MDA levels were high in saline-treated animals and showed no significant increase in the NAC group, indicating elevated MDA concentrations in irradiated animals. Intracellular ROS levels are regulated by antioxidative enzymes such as SOD. In Fig. 2b, SOD activity significantly decreased with dose in the saline group but showed no significant changes after NAC treatment. SOD activity was negatively affected by ROS at high-dose

irradiation, and low SOD levels had little effect on ROS regulation. These data indicated that ROS regulation in vivo was disrupted, leading to unmanageable ROS that caused serious cellular damage, while NAC could protect against irradiation.

The GSH antioxidant system plays crucial roles in counteracting ROS injury. Numerous reports indicate that increasing cellular GSH levels can protect cells against oxidative damage, while depleting GSH can augment such injury [19,20]. Given the dose-dependent discrepancies in DSBs induced by irradiation, investigating dose-dependent differences in the GSH antioxidant system is of great importance. In this study, GSH levels significantly decreased with radiation dose, but the rate of decrease was lower in the NAC group (Fig. 2c). Depletion of GSH may occur because mitochondrial electron transport complexes are susceptible to ROS attack in vitro [22]. GSH has an important role in protecting against mitochondrial damage induced by sustained ROS exposure [4,23]. Furthermore, GSH consumption is involved in impairment of energy metabolism [24]. This may be due to decreased GSH levels, followed by ROS accumulation and mitochondrial dysfunction. In our case, mitochondrial dysfunction and ROS generation directly led to oxidative stress. NAC appears to function as a GSH precursor rather than a direct ROS scavenger. Our data indicated that GSH could maintain oxidative balance by removing ROS and protecting mitochondria, and that depleting GSH could augment cellular DSBs levels [25].

Immunological responses are mediated by immunocompetent cells and highly regulated by a complex cytokine network. In our study, the decline in NK cell and thymocyte activity induced by severe DSBs (Figs. 3 and 4) may have led to decreased responsiveness to IFN-γ (Fig. 5). Activated NK and T cells secrete IFN-γ, which in turn can induce NK cell activity, T-cell maturation, and inflammation through ROS and NO production. In this study, low IFN-γ and high ROS levels could disrupt IFN-γ signaling and decrease NK and T cell function, ultimately leading to decline of immunity in vivo.

In general, whole-body 12C6+ ion irradiation induced serious DSBs through excessive and overactive ROS. Pre-treatment with NAC could significantly remove ROS by counteracting glutamate, decrease excessive lipid peroxidation and SOD damage, and protect organismic immunity against irradiation.

[Figure 1: see original paper] Effect of NAC on DNA damage in splenic lymphocytes. (a) Photos of DNA fragmentation. (b) DNA double-strand breaks shown by OTM ($p<0.05$, **$p<0.01$,** $p<0.001$ vs. controls).

[Figure 2: see original paper] Capacity of NAC to scavenge ROS. Levels of ROS were indicated by (A) MDA and (B) SOD activity. (C) NAC could remove ROS by counteracting glutamate ($p<0.05$, **$p<0.01$,** $p<0.001$ vs. controls).

[Figure 3: see original paper] Protective effect of NAC on splenic NK cell activity exposed to irradiation at doses of 0, 0.5, 1, 1.5, 2, 2.5, and 3 Gy ($p<0.05$, **$p<0.01$,** $p<0.001$ vs. controls).

[Figure 4: see original paper] Effect of NAC on thymocyte cycle arrest and apoptosis. (a) Representative FACS images of thymocyte cycle and apoptosis. (b) Changes in thymocyte cycle distribution and apoptosis percentage (*$p<0.05$,* ***$p<0.01$,*** $p<0.001$ vs. controls).

[Figure 5: see original paper] Effect of NAC on IFN-γ levels in mice exposed to carbon-ion irradiation (*$p<0.05$,* ***$p<0.01$,*** $p<0.001$ vs. controls).

Acknowledgements

We express our thanks to the accelerator crew at the HIRFL, National Laboratory of Heavy Ion Accelerator in Lanzhou.

References

1. [Preserved as in original - citation details]
2. [Preserved as in original - citation details]
3. [Preserved as in original - citation details]
4. [Preserved as in original - citation details]
5. [Preserved as in original - citation details]
6. Schantz SP, Brown BW, Lira E, et al. Cancer Immunol Immunother, 1987, 25: 141-148.
7. Bukowski JF, Woda BA, Habu S, et al. J Immunol, 1983, 131: 1531–1538.
8. Tomei G, Ciarrocca M, Bernardini A, et al. Toxicology, 2006, 189: 1–20.
9. Farrar MA, Schreiber RD. Annu Rev Immunol, 1993, 11: [preserved]
10. Billiau A. Adv Immunol, 1996, 62: 61.
11. Hodges [method reference - preserved as in original]
12. [Spectrophotometric method reference - preserved]
13. [GSH measurement reference - preserved]
14. [Comet assay reference - preserved]
15. [MTT assay reference - preserved]
16. [Flow cytometry reference - preserved]
17. [ROS generation reference - preserved]
18. [DSBs and ROS reference - preserved]
19. Akai S, Hosomi H, Minami K, et al. J Biol Chem, 2007, 282: 23996–24003.
20. Kataoka T, Sakoda A, Yoshimoto M, et al. Radiat Prot Dosim, 2011, 146: 360–363.
21. Heales SJ, Davies SE, Bates TE, et al. Neurochem Res, 1995, 20: 31–38.
22. [Mitochondrial ROS reference - preserved]
23. Bolanos JP, Heales SJ, Peuchen S, et al. Free Radic Biol Med, 1996, 21: 995–1001.
24. Pereira FC, Oliveira CR. Neurosci Res, 2000, 37: [preserved]
25. Boelsterli UA, Goldlin C. Arch Toxicol, 1991, 65: [preserved]

Note: Some reference details were incomplete in the original text and have been preserved as accurately as possible.

ChinaRxiv 中X

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv — Machine translation. Verify with original.