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Dose response of micronuclei induced by combination radiation of α-particles and γ-rays in human lymphoblast cells

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ABSTRACT

Combination radiation is a real situation of both nuclear accident exposure and space radiation environment, but its biological dosimetry is still not established. This study investigated the dose–response of micronuclei (MN) induction in lymphocyte by irradiating HMy2.CIR lymphoblast cells with α-particles, γ-rays, and their combinations. Results showed that the dose–response of MN induced by γ-rays was well-fitted with the linear-quadratic model. But for α-particle irradiation, the MN induction had a biphasic phenomenon containing a low dose hypersensitivity characteristic and its dose response could be well-stimulated with a state vector model where radiation-induced bystander effect (RIBE) was involved. For the combination exposure, the dose response of MN was similar to that of α-irradiation. However, the yield of MN was closely related to the sequence of irradiations. When the cells were irradiated with α-particles at first and then γ-rays, a synergistic effect of MN induction was observed. But when the cells were irradiated with γ-rays followed by α-particles, an antagonistic effect of MN was observed in the low dose range although this combination radiation also yielded a synergistic effect at high doses. When the interval between two irradiations was extended to 4 h, a cross-adaptive response against the other irradiation was induced by a low dose of γ-rays but not α-particles.

1. Introduction

It is well known that radiation, especially high linear energy transfer (LET) radiation, is an important environment carcinogenic event. For example, α-particle has became a prominent public health concern because of its presence in the residential environment (*i.e.*, radon (^{222}Rn) gas) and occupational environment (*i.e.* medical imaging and cancer therapy) [1,2]. Recent studies have demonstrated a positive correlation between the occurrence of lung cancer and exposure to ^{222}Rn gas [3,4]. DNA damage induced by high LET radiation is much more serious than low LET radiation, for instance, low dose rate exposures to alpha emitters were 15–20 times more damaging than exposures to beta or gamma irradiation [5]. It has been known that the relative biological effect (RBE) of ^{210}Po alpha-particle could vary from 1.6 to 21, depending on the endpoint: about 21 for cell viability, 13 for decrease in live cell number, 5.3 for LDH release, but only 1.6 for clonogenic survival due to X-ray hypersensitivity of endothelial cells at low doses [6], and DNA repair rate in α-particle irradiated cells is much slower than that of a low LET irradiation such as X-rays [7].

With chromosome aberration and micronuclei (MN) formation as checkpoints, the biological dosimetry of γ-rays has been well-established and widely applied. But the biological dosimetries of α-particles and related combination radiations are largely not clear. Complex radiation may happen in many situations. For example, during a nuclear reactor accident, radioactive isotopes including ^{239}Pu, ^{137}Cs, and ^{60}Co can be released simultaneously. ^{239}Pu can emit α-particles whereas ^{137}Cs and ^{60}Co yields γ-rays, the victims of a nuclear accident will suffer from their combination irradiation. On the other hand, radiation risk for the astronaut in space flight has been paid great attention since human activity in space. Space radiation includes X-rays, high energy protons, helium ions, and high-energy heavy particles. Even though being protected, the astronaut could be inevitably irradiated with multi-sorts of particles in space, which may result in significant risks of carcinogenesis and degenerative diseases at a long time of space flight. It has been reported that different irradiations of low- and high-LET may have cross-adaptive or synergic effect on cell survival [8]. Brooks et al. demonstrated that both cell killing and the induction of MN were increased by the combined exposures of alpha-particles and X-rays compared with that predicted for separate exposures [9]. Although understanding the biological dosimetry of complex radiation in space is a big challenge in the near future, it may give us essential information in evaluating cancer risk of space flight.

Abbreviations: LET, linear energy transfer; MN, micronuclei; RIBE, radiation induced bystander effect; SVM, state vector model.

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At present, cancer risks at doses lower than those for which direct epidemiological observations available are obtained by a linear extrapolation from the higher doses [10]. There are a number of low dose phenomena that might modulate the biological effects such that a linear extrapolation might not truly reflect low dose risk. These include radiation induced bystander effect (RIBE), adaptive response, and potential radiation sensitive subgroups in the human population. They might impact on the dose response of DNA damage at low doses of ionizing radiation [11,12]. In this study, MN induction in lymphocyte is employed to analyze the dose response of DNA damage induced by α-particles, γ-rays, and their combinations. It was found that a bystander effect was involved in the dose response of α-particle irradiation and either synergistic or adaptive response could be observed in different combination exposures.

2. Materials and methods

2.1. Cell culture

Human B lymphoblast cells (HMy2.CIR) were purchased from Shanghai Cell Bank of China and maintained in IMDM medium (HyClone, Beijing, China) containing penicillin (100 U/ml), streptomycin (100 U/ml) and 10% fetal bovine serum (Gibco Invitrogen, Grand Island, NY, USA) in humidified atmosphere of 5% CO_2 in air at 37 °C.

For α-particle irradiation, cells were grown on a 2.5 μm thickness Mylar film based dish to allow the penetration of α-particles from the bottom of the dish. The Mylar film was coated with 150–300 kD polylysine (Sigma) overnight then the log-phase HMy2.CIR cells were seeded on the dish and cultured for 4 h so that the lymphoblast cells well attached on the film at the time of α-particle exposure.

2.2. Cell irradiation

A ^{241}Am α-particle plate source (Atom HighTech Co., Ltd., Beijing, China) and a ^{137}Cs γ-ray irradiator (Model the Gamma-cell 40, Nordion Company of Canada) were applied for the cell irradiation in random. The energy of α-particles emitted from ^{241}Am isotope is 5.48 MeV. It was detected that, after passing through 3 layers of Mylar-film and possible air layer between films, the energy of α-particles arriving at cells was 4.4 MeV. It was calculated by a TRIM program that the LET of 4.4 MeV α-particle in water-equivalent tissue was 100 keV/μm and thus the dose rate of α-particles was calculated to be 0.244 Gy/min. The dose rate of γ-rays was 0.8 Gy/min. The irradiation doses were chosen as 0.1, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5 Gy for γ-rays and 0.01, 0.025, 0.05, 0.1, 0.15, 0.2, 0.5, 0.75, 1 Gy for α-particles. For the combination radiation, the HMy2.CIR cells were firstly irradiated with 0.025, 0.1, 0.2, or 0.5 Gy α-particles and then immediately (with an interval less then 5 min) exposed to 0.25, 0.75, 1, 2, 3 or 4 Gy γ-rays; or the cells were firstly irradiated with 0.25, 0.75, 1, or 2 Gy γ-rays and then given 0.025, 0.1, 0.2, or 0.5 Gy α-particles immediately.

On the other hand, to investigate the possible adaptive response between different irradiations, an interval of 4 h was given between α- and γ-irradiation. It has been known that this interval time was ideal for the induction of adaptive response. Briefly, the cells were irradiated with 0.025 or 0.1 Gy of α-particles, after 4 h, they were challenged with 2 Gy of γ-rays; or the cells were triggered with 0.1 Gy of γ-rays, after 4 h, the cells were challenged with 0.2 and 0.5 Gy of α-particles.

2.3. Micronucleus assay

After irradiation, cell damage was evaluated by MN assay using the cytokinesis-block technique. Briefly, after irradiation, all cells were collected and then treated with 3 μg/ml cytochalasin-B (Sigma) for 30 h for low dose irradiated cells. Our pilot experiments showed that a high dose of α-particle irradiation could cause cell cycle arrest, delayed nuclear division, and reduced MN formation. Therefore, when the cells were irradiated with α-particles of a dose higher than 0.1 Gy, the CB treatment time was prolonged to 48 h so that the cells could overcome cycle arrest as far as possible and yielded enough MN. After the CB treatment, the cells were treated with hypotonic solution of 75 μM KCl for 20 min and fixed in methanol with acetic acid (9:1), dropped onto glass slide, stained with Giemsa solution (Sigma), and observed under a microscope (Olympus, Tokyo, Japan). MN were scored in at least 500 binucleated cells and the MN yield, Y_{MN}, was calculated as the ratio of the number of MN to the scored number of binucleated cells.

2.4. Statistical analysis

Data were obtained from 3 to 5 independent experiments with three replicates in each case and were presented as mean ± SE. Statistical significance was acceptable at the level of $P < 0.05$. Data analysis was performed using the software SPSS11.5 (SPSS Inc., Chicago, IL, USA) and the dose–response curves were fitted with the Origin software (OriginLab Co., Northampton, MA, USA).

3. Results

3.1. Dose response of MN induced by γ-rays

Fig. 1 illustrates the dose–responses of the yield of MN in HMy2.CIR lymphoblast cells irradiated by α-particles and γ-rays. As shown in Fig. 1A, the yield of MN induced by γ-rays was well fitted with a linear–quadratic model represented by $Y_{MN} = c + \alpha D + \beta D^2$, where D is radiation dose, c is the frequency of background MN without irradiation, and α and β are linear and quadratic coefficients, respectively. It can be seen that the HMy2.CIR lymphoblast cells had a very low background frequency of MN, only 5×10^{-4}. The yield of MN increased slightly when the γ-irradiation dose was lower than 0.25 Gy but it increased steeply afterwards.

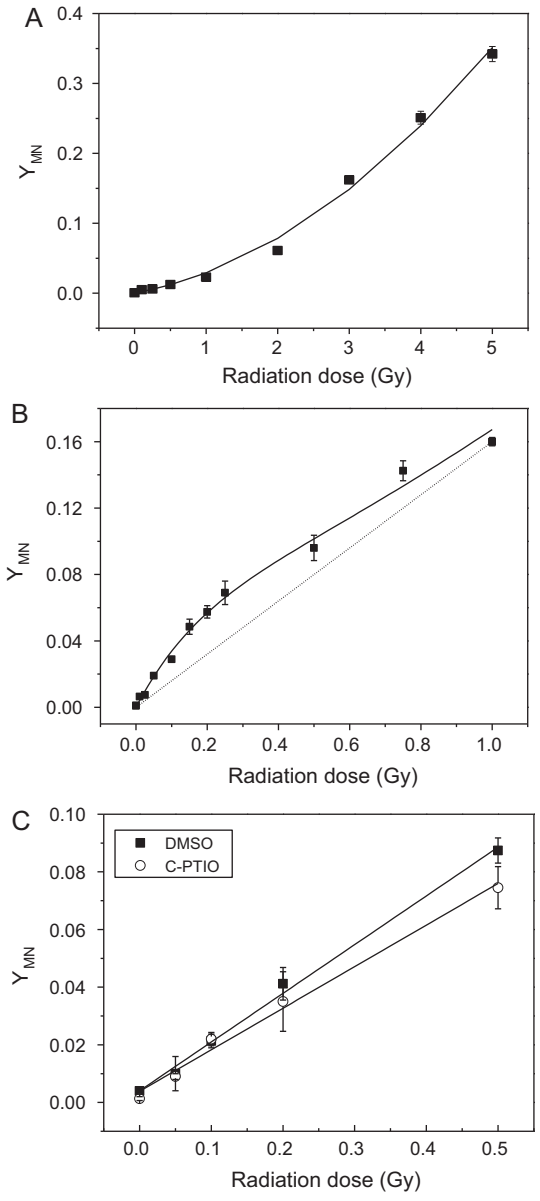

Fig. 1. Dose responses of MN in HMy2.CIR lymphoblast cells irradiated by different radiations. (A) Dose response of MN induced by γ-rays. The curve was fitted by a linear-quadratic model with an equation $Y_{MN} = c + \alpha D + \beta D^2$. (B) Dose response of MN induced by α-particles. The solid curve was fitted by a state vector model with an equation of $Y_{MN} = c + \alpha D + \sigma(1 - \exp(-\delta D))\exp(-\beta D)$. The dash line presented the dose response of the yield of MN induced by α-particles assuming no bystander effect. (C) Dose response of MN induced by α-particle radiation where the cells were pretreated with DMSO (■) and c-PTIO (●) before irradiation.