

Induction and Repair of HZE Induced Cytogenetic Damage

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Abstract

Wistar rats were exposed to high-mass, high energy (HZE) ⁵⁶Fe particles (1000 GeV/AMU) using the Alternating Gradient Synchrotron (AGS). The animals were sacrificed at 1-5 hours or after a 30-day recovery period. The frequency of micronuclei in the tracheal and the deep lung epithelial cells were evaluated. The relative effectiveness of ⁵⁶Fe, for the induction of initial chromosome damage in the form of micronuclei, was compared to damage produced in the same biological system exposed to other types of high and low-LET radiation. It was demonstrated that for animals sacrificed at short times after exposure, the tracheal and lung epithelial cells, the ⁵⁶Fe particles were 3.3 and 1.3 times as effective as ⁶⁰Co in production of micronuclei, respectively. The effectiveness was also compared to that for exposure to inhaled radon. With this comparison, the ⁵⁶Fe exposure of the tracheal epithelial cells and the lung epithelial cells were only 0.18 and 0.20 times as effective as radon in the production of the initial cytogenetic damage. It was suggested that the low relative effectiveness was related to potential for 'wasted energy' from the core of the ⁵⁶Fe particles. When the animals were sacrificed after 30 days, the slopes of the dose-response relationships, which reflect the remaining level of damage, decreased by a factor of 10 for both the tracheal and lung epithelial cells. In both cases, the slope of the dose-response lines were no longer significantly different from zero, and the r² values were very high. Lung epithelial cells, isolated from the animals sacrificed hours after exposure, were maintained in culture, and the micronuclei frequency evaluated after 4 and 6 subcultures. These cells were harvested at 24 and 36 days after the exposure. There was no dose-response detected in these cultures and no signs of genomic instability at either sample time.

KEYWORDS: Micronuclei, HZE, respiratory epithelial cells, rats.

1. Introduction and Methods

During prolonged space flights, there is a concern for the exposure to HZE particles and the potential for these particles to produce genomic instability [1] that could increase cancer risk [2]. Since most cancers originate in epithelial cells, it is important to study HZE induced changes in these cell types *in vivo*. The tracheal and lung epithelial cells were chosen for this study since the tracheal cells have been shown to be very resistant to the induction of cancer by high-LET radiation exposure, where as deep lung epithelial cells are sensitive [3].

Male Wistar rats, 4-6 weeks old were housed in AAALAC approved facilities at Brookhaven National Laboratory and exposed to 1000 MeV/AMU ⁵⁶Fe particles at graded doses of 0, 20, 50, 100, and 200 cGy. They were sacrificed either within 1-5 hours after the exposure or after 30 days of *in vivo* recovery and the tracheal and deep lung epithelial cells were isolated and placed in short term culture. The cells were prepared for evaluation of micronuclei using previously published methods [4]. Slides were coded and scored for the frequency of micronuclei in binucleated cells. Deep lung epithelial cells were further transferred in culture and were scored for micronuclei after passage 2, 4 and 6.

2. Results and Discussion

The results of the cytogenetic evaluations have been submitted for publication. Briefly, the slope of the dose-response relationship for induction of micronuclei/binucleated cell were 11.0×10^{-4} and 12.0×10^{-4} for the tracheal epithelial and deep lung epithelial cells, respectively. These were related to the frequency of micronuclei induced following exposure to either the low-LET radiation from ⁶⁰Co gamma rays (4) or the high-LET alpha particles (5) from inhalation of radon. It was demonstrated that for the tracheal epithelial cells and the lung epithelial cells the ⁵⁶Fe particles were 3.3 and 1.3 times as effective as ⁶⁰Co in production of micronuclei. The effectiveness for exposure to inhaled radon demonstrated that ⁵⁶Fe exposure of the tracheal epithelial cells and the lung epithelial cells were only 0.18 and 0.20 times as effective as radon in the production of the initial cytogenetic damage. It was suggested that the low relative effectiveness could be related to potential for 'wasted energy' from the core of the ⁵⁶Fe particles. Calculations indicated that up to 32 cells interact with the delta rays for every cell that is traversed by a primary particle. The data in the present paper suggest that much of the damage scored as micronuclei were induced by the delta rays.

To evaluate the loss of damage from these systems, animals were sacrificed after 30 days of recovery *in vivo*. The frequency of micronuclei/binucleated cell was plotted against the radiation dose and fit using linear-least squares methods. The equations that describe the data were:

Tracheal epithelial cells:

$$\text{Micronuclei/binucleated cell} = 0.01 + 1.2 \times 10^{-4} \text{ Dose}$$

Deep lung epithelial cells:

$$\text{Micronuclei/binucleated cell} = 0.03 + 7.0 \times 10^{-5} \text{ Dose}$$

These dose-response relationships reflect the remaining level of damage after 30 days of repair *in vivo*. The frequency of micronuclei/binucleated cell decreased by about a factor of 10 for both the tracheal and lung epithelial cells. This loss was much greater than observed in deep lung fibroblasts following exposure to high-LET radon exposure [6]. This suggests that the cells damaged by ^{56}Fe are more rapidly repaired or removed from these cell populations than from the deep lung fibroblasts.

Deep lung epithelial cells from animals that were sacrificed from 1-5 hours after exposure were maintained in long term culture to determine if genomic instability would be induced by the radiation exposures. These cultures were maintained up to six passages or about 36 days after the initiation of the cultures. No increase in the frequency of micronuclei was found in any of the cultures as a function of radiation dose. This suggests that at this time post

exposure, using micronuclei as the genetic endpoint, there was no indication of radiation induced genomic instability.

In summary these data suggest that: i) exposure to ^{56}Fe particles is not highly effective in production of micronuclei, ii) that the cells damaged by ^{56}Fe are rapidly eliminated from the exposed tissue, iii) that the deep lung epithelial cell system, with the current protocol, did not detect evidence of genomic instability.

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