

Induction of Chromatin Damage and Distribution of Isochromatid Breaks in Human Fibroblast Cells Exposed to Heavy Ions

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The frequency of chromatid breaks and the distribution of isochromatid breaks were measured in G2-phase normal human fibroblasts prematurely condensed a short time after exposure to low- or high-LET radiations. The average number of isochromatid breaks from a single particle traversal increased with increasing LET values, while the average number of chromatid-type breaks appeared to reach a plateau. The distribution of isochromatid breaks after high-LET iron particles exposure was overdispersed compared to γ -rays, indicating that a single iron particle traversal through a cell nucleus can produce multiple isochromatid breaks.

INTRODUCTION

During space flight, crewmembers are exposed to a mixed radiation field that includes charged particles of solar origin (protons and electrons) and galactic radiations (protons 85%, α particles 14% and heavy ions 1%). Although heavy ions contribute only a small percentage of the overall dose, the biological effect could be significant because heavy ions are highly charged and very densely ionizing. Heavy ions are associated with high linear energy transfer

(LET) and have been shown to be more effective than low-LET radiations for the induction of many endpoints such as cell killing^{1–4}, mutation^{5–7}, and transformation⁸). An accurate assessment of the biological effects of heavy-ion exposure is necessary to predict and reduce the associated health risks for exposed individuals.

Chromosome aberrations are the most sensitive biological indicator of absorbed dose⁹, and since there is substantial evidence relating chromosome aberrations to carcinogenesis¹⁰ and cell killing¹¹, cytogenetic analysis is, therefore, also regarded as a reliable method for assessment of radiation risk^{12,13}). Classical cytogenetic studies involve assessing chromosome damage in metaphase cells at first division after exposure. However, for analysis of high-LET effects, mitotic cells present several problems. It is well known that radiation induces delays in cell-cycle progression that influence the frequency of aberrations detected in mitosis, and that these delays are LET and dose dependent^{14–17}). Therefore, analysis of mitotic cells harvested at one time point may lead to an underestimation of radiation-induced damage, especially in cells exposed to high-LET radiations^{17,18}). With the recently developed chemically induced premature chromosome condensation (PCC) technique, chromosomes can be condensed in different phases of the cell cycle^{19,20}) and it is possible to assess chromosome damage in G2-phase, avoiding the cell-cycle selection problems associated with metaphase analysis. With chemically induced PCC technique it is also possible

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sible to measure the initial number of chromosome damages shortly after exposure. In a previous study we used the PCC technique to measure the induction of G2-chromatid breaks and the repair kinetics of these breaks in normal human fibroblasts after exposure to various types of heavy ions, and we conclude that an increased induction of isochromatid break is a signature of high-LET irradiation^{21–23}. In the study presented here, we show the average number of chromatid-type breaks, isochromatid breaks, and total breaks from a single particle traversal, along with the distribution of isochromatid breaks following high-LET irradiation.

MATERIALS AND METHODS

Irradiation

Normal human fibroblasts AG1522 cells (obtained from the NIA Cell Repository, Camden, NJ, USA) were grown in α -MEM medium supplemented with 10 % calf serum at 37°C in 5 % CO₂ atmosphere with 95 % humidification. AG1522 cells were grown in T-25 flasks and exponentially growing monolayer cells were irradiated with γ -rays (¹³⁷Cs) source at Baylor College of Medicine, Texas Medical Center, Houston, Texas in USA. Doses were verified through the use of an ionizing chamber dosimetry. Irradiation with heavy ions was performed at Heavy Ion Medical Accelerator Chiba (HIMAC) in Japan and Brookhaven National Laboratory, New York in USA. For heavy ions, doses were measured with a calibrated parallel-plate ionization chamber²⁴. The beam energy of silicon particles was 490 MeV/u corresponding to an average LET of 55 keV/ μ m. The beam energy of iron particles were 600 MeV/u, 500 MeV/u and 200 MeV/u, corresponding to an average LET of 140 keV/ μ m, 185 keV/ μ m and 440 keV/ μ m, respectively. The beam energy of carbon particles was 290 MeV/u, corresponding to an average LET of 13 keV/ μ m. Carbon particles accelerated to 290 MeV/u were shielded with 147 mm water-equivalent absorber to obtain a higher LET value. The average LET of the shielded carbon particles was 80 keV/ μ m.

Chromosome Aberrations

Chromosomes were forced to condense prematurely using calyculin A (Wako Chemicals, Osaka, Japan). In order to measure the initial G2-chromatid breaks, 50 nM calyculin A was added to the growth medium 5 minutes before irradiation and cells were further incubated for 25 minutes at 37°C as described elsewhere^{21,22,25}. Cells were then swollen in 75 mM KCl for 20 minutes at 37°C, and fixed with methanol: glacial acetic acid (3:1 vol./vol.). A final wash and fixation in fresh fixative was completed before dropping cells

onto a glass slide and air-drying. To determine the number of initial chromatid breaks, chromatid-type breaks and isochromatid breaks were scored separately. Chromatid discontinuity, misalignment of the segment distal to the lesion was classified as one chromatid break. The yield of isochromatid breaks was measured from the excess number of chromosomes (> 46) observed. Since one isochromatid break results from the breakage of both chromatids, we scored one isochromatid break as two chromatid breaks. The total number of breaks was calculated by summing the yields of chromatid-type breaks and isochromatid breaks. At least, 40 cells were analyzed in this study.

RESULTS AND DISCUSSION

Previously, we reported that the dose response curves for total chromatid breaks (chromatid-type plus isochromatid breaks) and chromatid-type breaks were linear regardless of radiation type, and that the dose response curves for isochromatid-type breaks were linear for high-LET radiation but linear quadratic for low-LET radiations²². The average frequency of each type of break induced by a single particle traversal was determined from the linear component (breaks/Gy) of dose response curves and the cross sectional area of AG1522 cells in G2 cell cycle phase ($242 \pm 4 \mu\text{m}^2$; mean \pm SE)²². The mean particle number traversing a nucleus (N) can be determined from particle fluence (F) using the equation²⁶

$$N = 6.25 \times A (\mu\text{m}^2) \times D (\text{Gy}) / \text{LET} (\text{keV} / \mu\text{m})$$

Where A is a cross-sectional area (μm^2) and D is dose (Gy).

The linear component (breaks/Gy) of each type of break is shown in table 1. Using the equation listed above, the average yields of chromatid-type breaks, isochromatid

Table 1. The average number of breaks/Gy for chromatid-type, isochromatid-type and total breaks.

Radiation	LET (keV/ μ m)	Chromatid-type (Breaks/Gy)	Isochromatid-type (Breaks/Gy)	Total Breaks (Breaks/Gy)
Carbon	13	9.10 \pm 0.31	0.74 \pm 0.32	10.94 \pm 0.72
Silicon	55	18.38 \pm 0.23	5.08 \pm 0.40	22.25 \pm 1.1
Carbon	80	13.81 \pm 0.22	7.88 \pm 0.26	22.24 \pm 0.86
Iron	140	8.6 \pm 0.83	7.62 \pm 0.14	16.30 \pm 1.85
Iron	185	9.29 \pm 0.05	7.64 \pm 0.08	17.35 \pm 0.92
Iron	440	4.99 \pm 0.28	7.32 \pm 0.46	12.20 \pm 0.56

The number of breaks/Gy for each type of breaks was determined from the linear component of dose response curves (17). One isochromatid break is estimated to be equal to two breaks.

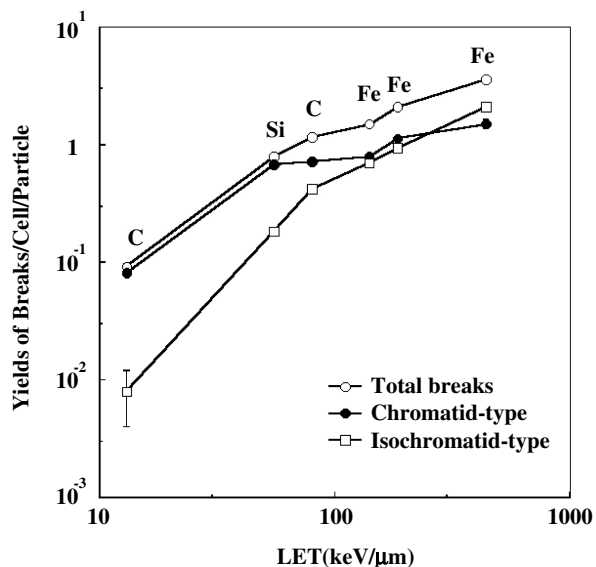


Fig. 1. The yields of chromatid-type breaks, isochromatid breaks and total breaks from a single particle traversal as a function of LET. The yield of one isochromatid break was regarded as equal to two chromatid breaks. The error bar represents S.E. of the mean value.

breaks, and total breaks from a single particle traversal were calculated and the results are shown in Fig.1. The results demonstrate a LET dependence for each type of break. On the log-log plot, the average yields of isochromatid breaks increased continuously with increasing LET values. There is an average of 1 isochromatid break (= two breaks) per particle traversal of the cell nucleus at an LET of 440 keV/ μm . In contrast, the average yields of chromatid-type breaks increased up to 55 keV/ μm where it appeared to reach a plateau. The average yields of isochromatid breaks were lower

than chromatid-type breaks for LET values up to 145–185 keV/ μm , whereas the yield of isochromatid breaks were higher than chromatid-type breaks at 440 keV/ μm .

Fig. 2 shows the distributions of isochromatid breaks among cells exposed to similar doses of γ -rays or iron particles at two energies with LET of 185 keV/ μm and 440 keV/ μm respectively. To evaluate the distribution of the number of isochromatid breaks in irradiated cells, the relative variance (s^2/y) was determined from the measured value of the mean value (y) and the variance (s^2). The relative variance appears to increase with increasing LET values, suggesting that overdispersion of isochromatid break distribution occurs after higher-LET radiations. Similar conclusions were drawn from previous G0/G1 PCC studies^{27,28}, where the authors reported that the number of PCC fragments per cell became progressively overdispersed with increasing LET. The alteration in the pattern of break dispersion could be explained by the different energy deposition for low- and high-LET radiations. As the LET of a particle increases, energy deposition can no longer be considered random; high energy is deposited close to the trajectories of individual high-LET particle tracks. A single particle traversal of high-LET radiations could produce multiple isochromatid breaks, resulting in the overdispersion of breaks in cells exposed to high-LET radiations.

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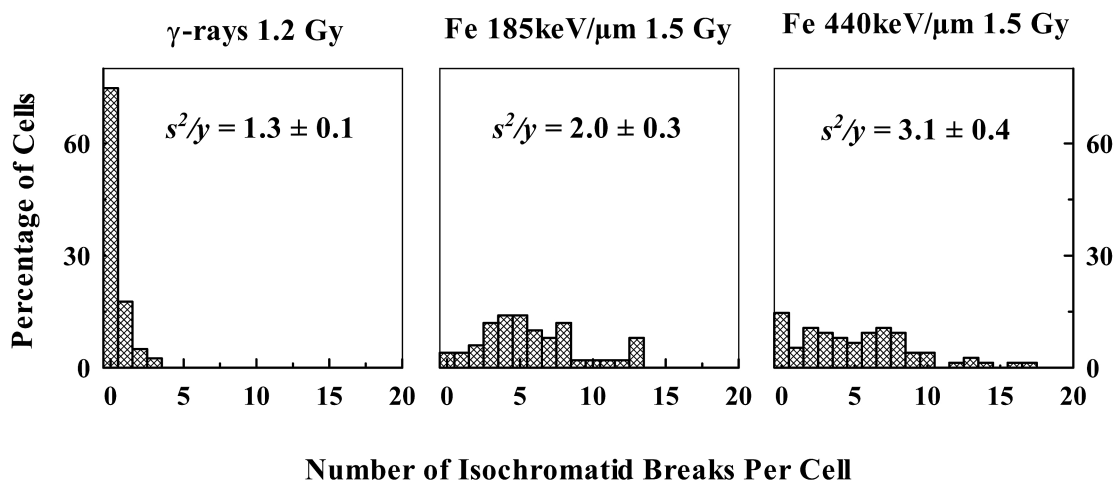


Fig. 2. Distribution of isochromatid breaks in cells exposed to similar doses of radiations of different LET. The relative variance (s^2/y) and the standard error (S.E.) are shown in each plot.

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