Induction of Sister Chromatid Exchanges by Extremely Low Doses of \( \alpha \)-Particles

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Abstract

The induction of sister chromatid exchanges (SCE) was examined in Chinese hamster ovary cells irradiated in the G1 phase of the cell cycle with \( \alpha \)-particles from a plutonium-238 source. A significant increase in the frequency of SCE occurred with doses as low as 0.31 mGy (31 millirads). Although 30\% of the cells showed an increased frequency of SCE at this dose, less than 1\% of cell nuclei were actually traversed by an \( \alpha \)-particle. A dose of approximately 2.0 Gy was necessary to produce a similar increase in SCE by X-rays. These results indicate that genetic damage may be induced by low doses of \( \alpha \)-radiation in cell nuclei not actually traversed by an \( \alpha \)-particle. This phenomenon may have important implications in the estimation of risks of such exposures.

Introduction

In order to produce genetic damage, it has been generally assumed that an \( \alpha \)-particle must traverse the nucleus of a cell producing DNA damage. This damage may cause gene mutations or chromosomal changes in surviving daughter cells. Recently, however, Kadhim et al. (1) showed that chromosomal aberrations appearing in hematopoietic cells may not be a direct consequence of such DNA damage. Rather, \( \alpha \)-irradiation induced a type of transmissible genetic instability that led to the occurrence of aberrations among progeny cells after many generations of replication. In the present investigation, we have studied the induction of SCE in cells synchronized in G1 and exposed to very low doses of \( \alpha \)-particles from a plutonium-238 source. We show that a significantly increased frequency of SCE is induced by doses in the range of those associated with occupational exposures to radon in homes. SCE were elevated in the nuclei of many cells that were not actually traversed by an \( \alpha \)-particle.

Materials and Methods

CHO cells of clone 8 were cultured in Eagle's MEM supplemented with 10\% calf serum heat inactivated for 30 min at 56°C, penicillin (50 units/ml), and streptomycin (50 \( \mu \)g/ml). Synchronization of the cell population in the G1 phase of the cell cycle was accomplished by the isoleucine depletion method previously described by Tobey and Ley (2). Briefly, 2 \( \times \) 10\(^4\) cells were seeded on 1.5-\( \mu \)-m-thick Mylar base dishes coated with collagen to facilitate cell attachment. When the growth of the cultures reached 30 to 40\% confluence, the culture medium was removed and replaced with isoleucine-deficient MEM containing 5\% of 3\% dialyzed fetal bovine serum, and the cultures were incubated at 37°C for 30 h. Approximately 10\% of the cells were in the S phase at this time as determined by autoradiography following a 15-min pulse label with \( \text{[H]} \) thymidine.

Irradiations were carried out from below through the Mylar base dishes with 3.7 MeV \( \alpha \)-particles derived from plutonium-238; a detailed description of this source and dosimetric measurements will be presented elsewhere.\(^4\) It was fitted with a photographic shutter to allow the accurate delivery of the specified radiation dose at a dose rate of 0.147 Gy per min with exposure times of a fraction of a second. After irradiation, the medium in each dish was changed to complete MEM containing 10\(^{-5}\) M bromodeoxyuridine and cultured at 37°C for two rounds of cell replication. Colcemid (0.2 \( \mu \)g/ml) was added to the culture for 4 h prior to fixation. Air drying and the differential staining of SCE were carried out by the fluorescence plus Giemsa technique (3). The number of SCE observed in a total of 50 to 100 cells were scored in each experiment for each data point; based on a modal chromosome number of 21, the data are presented as the mean number of SCE per chromosome.

Results and Discussion

The absorbed radiation dose and mean number of \( \alpha \)-particle tracks per nucleus are shown in Table 1 for the various exposure times used in these experiments. As can be seen, very few nuclei (1\% or less) were actually traversed by an \( \alpha \)-particle at these doses. The dose-response curve for the induction of SCE by \( \alpha \)-particles is shown in Fig. 1. These data represent the results of 3 to 5 separate experiments. The background frequency of SCE for each particular experiment was subtracted from the frequency observed in each radiation group in order to obtain the induced frequency. The data points shown represent the mean \( \pm \) 1 SEM of the results of these individual experiments. The mean background frequency, based on scoring 520 cells in 7 experiments, was 0.342 \( \pm \) 0.013. As the modal chromosome number of these cells was 21, this represents about 7 SCE per cell.

As can be seen in Fig. 1, the frequency of SCE was markedly elevated by an exposure time of 0.125 s (0.31 mGy) and reached plateau with 1-s exposure (2.45 mGy). The frequency of SCE at the plateau was about 1.4 times the background level. A comparison of the distributions of SCE frequencies among individual cells irradiated with 0, 0.31 mGy, or 2.45 mGy is shown in Fig. 2. The open bars in Fig. 2, B and C, represent the background frequencies of SCE based on the distribution in Fig. 24. The hatched bars represent SCE induced by the \( \alpha \)-particle irradiation. When the distribution was analyzed among the individual cells, 30\% and 43\%, respectively, of the cells irradiated with 0.31 or 2.45 mGy showed enhanced levels of SCE. Approximately 13\% of cells irradiated with \( \alpha \)-particles contained more than 0.6 SCE per chromosome; cells with this frequency of SCE were rarely seen in nonirradiated cell populations. Based on the data shown in Table 1, we estimate that about 0.1 to 0.5\% of the cell nuclei were actually traversed by an \( \alpha \)-particle at these dose levels.

The maximum enhancement ratio of 1.4 observed in these experiments for cells irradiated in G1 with \( \alpha \)-particles is consistent with previous findings in mouse (4) and other Chinese

\(^4\) N. F. Metting and A. Koehler, manuscript in preparation.
Table 1  Dosimetry for α-particle irradiation

<table>
<thead>
<tr>
<th>Exposure time (s)</th>
<th>Absorbed dose (mGy)</th>
<th>Mean no. of α-tracks/nucleus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.067</td>
<td>0.16</td>
<td>0.0004</td>
</tr>
<tr>
<td>0.125</td>
<td>0.31</td>
<td>0.0007</td>
</tr>
<tr>
<td>0.250</td>
<td>0.61</td>
<td>0.0014</td>
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<tr>
<td>0.500</td>
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<td>0.0028</td>
</tr>
<tr>
<td>1.000</td>
<td>2.45</td>
<td>0.0056</td>
</tr>
<tr>
<td>2.000</td>
<td>4.90</td>
<td>0.0112</td>
</tr>
</tbody>
</table>

*CR-39 track etch plastic was used for measurements of particle fluence and field uniformity. The dose rate was 0.0054 α-track/mm²/min, and the average size of the nucleus was 62.2 μm²; yielding 0.366 tracks/nucleus/min.

![Graph showing SCE induction by α-particle irradiation](image)

Fig. 1. Induction of SCE by irradiation with plutonium-238 α-particles. Induced frequencies of SCE were obtained by subtracting background from observed frequencies in each experiment (see text). χ² test yielded P < 0.005 between control and cells irradiated for 0.125 to 2.0 s (0.31 to 49 mGy). Points, mean of 3 to 5 individual experiments representing a score of a total of 375 cells for each (except 2 s = 295 cells, and points without bars represent single experiments); bars, SEM.

Hamster cell lines (5). In all of these cell lines, a comparable elevation in SCE required approximately 1.0 to 2.0 Gy of X-irradiation. Thus, the relative biological effectiveness is very high (>100) for this end point. This is consistent with earlier findings of the induction of SCE in resting human lymphocytes (6) and with the conclusions of Kadhim et al. (1) in mouse hematopoietic cells. In these latter cases, however, no effect could be demonstrated with X-irradiation, suggesting a relative biological effectiveness approaching infinity.

The significance of SCE in mammalian cells is not clear, though it does represent a type of genetic change within the cell. Clearly, however, the kinetics of the induction of SCE differ from those for chromosomal aberrations. In a previous study (5), we observed a linear increase in the frequency of chromosomal aberrations in CHO K-1c cells irradiated with doses of 20 to 300 mGy. As the aberration frequencies observed are much lower than those for SCE, the findings were consistent with an increased frequency of aberrations occurring in cells traversed by one or more α-particles. Interestingly, however, most of the cells containing aberrations following irradiation with 20 to 60 mGy survived; in the present study, no evidence of cell killing was observed at any of the doses studied (up to 4.9 mGy).

The mechanism by which elevated frequencies of SCE occur in irradiated cells in which the nucleus incurs no direct interaction with an α-particle is not clear. It is possible that communication among cells may occur at the molecular level through autocrine signaling processes, perhaps through activation of signal transduction pathways following α-particle interactions with the cell membrane or cytoplasm. Peroxidized unsaturated fatty acid products have been found in subcellular fractions of rat liver immediately after irradiation; the amount of these products increased markedly during postirradiation incubation at 37°C (7). Asaoka et al. (8) reported that unsaturated fatty acids and diacylglycerol activate some members of the PKC family of enzymes at the basal level of Ca²⁺ concentration. PKC plays a critical role in cell growth, and PKC activated by phorbol ester tumor promoters can produce a cascade of events including O₂⁻ free radicals and alterations in gene expression. Frenkel et al. (9) reported that the tumor promoter 12-O-tetradecanoyl phorbol-13-acetate induced radiation-like DNA base damage in human polymorphonuclear lymphocytes. It has been shown that phorbol ester tumor promoters can induce SCE in cells at concentrations which produce no other measurable genotoxic effects (10, 11).

SCE are thought to reflect the activity of recombinational processes (10), and there is evidence that such recombinational activity may be induced in both yeast and human cells by ionizing radiation (12, 13). This activity might be turned on by signals arising outside of the nucleus (14). Long-lived free radicals such as superoxide (O₂⁻) may be produced when α-particles interact with cell membranes. A cascade of free radical reactions may then occur including the production of hydrogen peroxide (H₂O₂) and organic peroxides that results eventually in the production of hydroxyl radicals within the nucleus causing DNA damage. It has been proposed that the clastogenic effects of phorbol ester tumor promoters are mediated through the production of O₂⁻ (15). Evidence for an indirect effect of irradiation in producing SCE has also arisen from studies with heterokaryons following fusion of irradiated and nonirradiated mouse and CHO cells (16).

Though the mechanism remains unclear, the fact that genetic changes can be induced in cells by such low levels of exposure to α-radiation offers further evidence that the risk to occupationally exposed individuals may not be easily extrapolated from epidemiological data for low LET radiation (1). These findings may also have important implications for exposure standards for the general population. The dose range yielding

![Graph showing SCE distribution among individual cells irradiated with plutonium-238 α-particles](image)

Fig. 2. Distribution of frequencies of SCE among individual cells irradiated with plutonium-238 α-particles. A, nonirradiated controls; B, 0.125 s (0.31 mGy); C, 1.0 s (2.45 mGy). The hatched bars in the histograms represent α-particle-induced SCE while the open bars represent background frequencies of SCE based on the distribution observed in nonirradiated cells (4). The results are from a single experiment (No. 220-6), but similar results were observed in all other experiments.
significantly increased frequencies of SCE in the present study (0.31 to 4.9 mGy or 31 to 490 millirads) is well within the range of exposure reported to occur from radon in many homes.

References