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Indications of Repair of Radon-Induced Chromosome Damage in Human Lymphocytes: An Adaptive Response Induced by Low Doses of X-Rays

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Naturally occurring radon is a relatively ubiquitous environmental carcinogen to which large numbers of people can be exposed over their lifetimes. The accumulation of radon in homes, therefore, has led to a large program to determine the effects of the densely ionizing α particles that are produced when radon decays. In human lymphocytes, low doses of X-rays can decrease the number of chromatid deletions induced by subsequent high doses of clastogens. This has been attributed to the induction of a repair mechanism by the low-dose exposures. Historically, chromosome aberrations induced by radon have been considered to be relatively irreparable. The present experiments, however, show that if human peripheral blood lymphocytes are irradiated with low doses of X-rays (2 cGy) at 48 hr of culture, before being exposed to radon at 72 hr of culture, the yield of chromatid deletions induced by radon is decreased by a factor of two. Furthermore, the numbers of aberrations per cell do not follow a Poisson distribution but are overdispersed, as might be expected because high-linear energy transfer (high LET) α particles have a high relative biological effectiveness compared to low-LET radiations such as X-rays or γ rays. Pretreatment with a low dose of X-rays decreases the overdispersion and leads to a greater proportion of the cells having no aberrations, or lower numbers of aberrations, than is the case in cells exposed to radon alone. It therefore appears that the putative chromosomal repair mechanism induced by low doses of sparsely ionizing radiation is also effective in reducing cytogenetic damage induced by α particles, which hitherto had been thought to be relatively immune to repair processes.

Introduction

Environmental carcinogens most often cause their effects through the genetic damage they inflict in somatic cells. Although these agents can induce point mutations, most of the damage comprises larger chromosomal effects such as deletions, translocations, and inversions (i.e., chromosomal aberrations). These gross chromosomal effects are the result of the breakage of chromosomes and the subsequent repair, or rejoining, of the broken ends to form abnormal chromosomes. In human lymphocytes, it has been found that preexposures of the cells to very low doses of radiation from incorporated isotopes (*1–4*), exogenous X-rays (*5–8*), or even chemical clastogens (*9,10*) can induce an adaptive response whereby the cells become refractory to the induction of aberrations by subsequent exposures to high doses of these agents. Low doses of X-rays can also lead to a reduction in point mutations induced by high doses of X-rays (*11*). This adaptation has been attributed to the induction of a repair mechanism that, if in place at the time of the high-dose challenge, reduces the number of broken chromosome ends that can take part in aberration formation.

Recently there has been a great deal of concern about radon, a relatively ubiquitous, naturally occurring environmental carcinogen. Radon, which can accumulate in houses and lead to high cumulative doses in the population, is particularly insidious because its decay results in the emission of high-LET α particles with a high relative biological effectiveness (RBE), and deposition of radon daughter products within the lung can lead to carcinogenic doses to target cells and tissues of the respiratory tract (*12*). Part of the high RBE has been attributed to a

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relative lack of repair or rejoining of α-particle-induced chromosome breaks (*13*).

The present experiments, therefore, were undertaken to see if the putative repair mechanism induced by low doses of X-rays would be operative on the chromosome damage induced by the very high-LET α particles from radon. The experiments show that the adaptive response induced by low doses of X-rays also affects the chromosomal breaks produced by α particles.

Materials and Methods

Human lymphocytes were obtained from the venous blood of healthy adult donors. The blood was drawn into heparinized (sodium heparin) vacutainer tubes. Whole blood (0.5 mL) was then added to 4.5 mL of RPMI 1640 medium containing 10% fetal calf serum, 2 mM glutamine, 100 units/mL penicillin, 100 mg/mL streptomycin, and 2% phytohemagglutinin M (Gibco, Grand Island, NY). The blood was cultured at 37°C in 1-oz glass prescription bottles. Colcemid (0.1 mL, final concentration 2×10^{-7} M) was added to each culture 2 hr before fixation. The cells were collected by centrifugation, exposed to a 0 075 M KCl solution for 16 min to spread the chromosomes, and then fixed in methanol-acetic acid (3:1). Cytological preparations were made by dropping cells onto wet slides and staining with Giemsa (Gurr's R66). Slides were scored for chromatid and isochromatid deletions.

Cells irradiated with X-rays were exposed at 37°C to 250-kVp X-rays from a Norelco Model MG300 X-ray unit, 10 mA, half-value layer 1.06 mm Cu. For positive control experiments on adaptation by X-rays, the cells were pretreated with 2 cGy of X-rays (20 cGy/min) at 48 hr of culture, then challenged with a high dose (150 cGy at 90 cGy/min) of X-rays at 72 hr of culture, and fixed 6 hr later.

Radon gas (from 0.7 Ci of $^{226}RaCl_2$) was mixed with 100% CO_2 to make a 5% CO_2 aerosol. This flowed through a sterile filter and then passed over 50 mL of cell culture medium. Approximately 4 hr after the gas flow was initiated, radon and its daughters reached a steady state in the medium. The flow rate of the radon gas and the volume of the medium were adjusted to give a dose rate of approximately 15 cGy/hr. For studies on the adaptive response, two different cell cultures were simultaneously exposed to α particles. One culture was pretreated with a low dose of X-rays (2 cGy) at 48 hr, and the other was not. The two spinner flasks were connected in parallel with the radon CO_2 gas mixture, and the gas flow was adjusted in each flask to equalize the concentrations of radon gas. After the steady state was reached, blood cells were collected by centrifugation, and, at 72 hr, 2 mL of the resulting concentrated cell suspension was injected into the flasks by way of a sterile syringe inserted into the tubing of the closed system. At appropriate times, cells were removed from the system, washed to remove the culture medium containing radon and daughter products, resuspended in RPMI 1640 medium, and cultured for various periods of time to allow the cells to reach metaphase, where they could be scored for chromosome aberrations. The doses in the two flasks were within 7% of each other, as determined by measurements and calculations made after the treatments. For more details see Wolff et al. (*14*), on which the present paper is based.

Scintillation spectrometry (*15*) was used to determine the α-particle activity in the exposure medium. The scintillation and counting parameters were calibrated by using a $RaCl_2$ standard. α-Particle activity was measured when the cells were first introduced into the closed exposure system and thereafter at each time samples of cells were withdrawn. Furthermore, serial measurements of the total activity were made of each sample. The total number of α particles emitted by radon (^{222}Rn) and its daughters (^{218}Po, ^{214}Po) per unit volume of culture medium was then calculated for each interval of time by solving a set of differential equations for the growth and decay of radon daughters. The doses received by the cell nuclei were calculated theoretically from the dimensions of the nuclei as well as the fluence and stopping power of the α particles as a function of energy. Corrections were made for the dose component due to radioactivity associated with the cells by using a standard curve derived from experiments with radon-exposed Chinese hamster ovary cells.

Student's *t*-test was used to test the differences between the expected and observed number of aberrations. The chi-square test was used to test the goodness of fit to the Poisson distribution. The method of Cohen (*16*) was used to estimate the mean number of deletions per cell for the conditional, or zero-truncated, Poisson distribution. In the chi-square tests, the data were grouped so that no expected value was < 5.

Results

With successive fixation times after exposure to 18 cGy of radon, the yield of chromosomal aberrations increased (Fig. 1). Approximately 80% of the aberrations observed were chromatid deletions. Dicentric and ring chromosomes were observed in some cells at later harvest times, indicating that cells analyzed at these times had been in G_1 when they were irradiated. The numbers of chromatid aberrations increased linearly with the dose of α particles from radon in lymphocyte cultures exposed to various doses and harvested at a single time after exposure. The data for cells fixed 11 hr after exposure are presented in Figure 2.

These results indicate that, even though the yield of aberrations is linearly related to dose, if cells are analyzed at a fixed time after radon exposure, the yield also increases with each successive fixation time. Consequently, in studies of the effects of low doses of X-rays on radon-induced chromosomal damage, several fixations must be performed to ensure that the observed effects are not merely the result of sampling cells from different parts of the cell cycle with differential sensitivity to radiation. The results of an experiment performed to see if a preexposure to a low dose of X-rays would lead to a decrease in aberrations induced by a subsequent exposure to α parti-

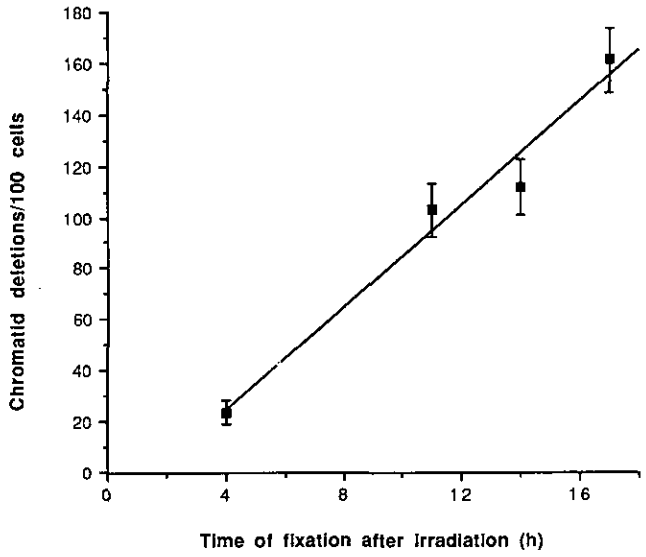

FIGURE 1. *Relation of chromatid deletions to time after exposure to 18* cGy of radon. One hundred cells per point were scored, except at 14 hr, when 200 cells were scored. Data from Wolff et al. (*14*).

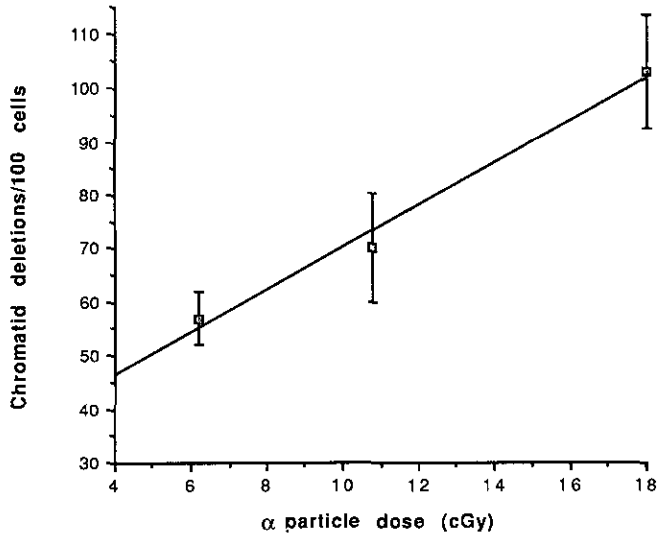

FIGURE 2. Relation of chromatid deletions to dose of α particles from radon. One hundred cells per point were scored from fixations made 11 hr *after exposure. Data from Wolff et al. (14).*

cles showed that, although the yield of chromatid aberrations increased with time after each successive fixation, the number of aberrations in those cultures pretreated with 2 cGy of X-rays was significantly lower at each fixation time (Table 1). Cells pretreated with a low dose of X-rays contained approximately one-half the expected number of aberrations at all harvest times.

Mitotic indexes calculated after the various treatments (Table 2) provide a further indication that stage-sensitivity differences could not account for the decrease in aberrations obtained when the cells were preexposed to X-rays. The time of fixation did not affect the mitotic indexes in the control (unirradiated) cultures or in those exposed to 2 cGy of X-rays alone. As expected, however, exposure to radon did decrease the number of mitotic cells found at the various fixation times. At any given fixation time, there were no significant differences in the mitotic indexes of cells exposed to radon alone or to X-rays plus radon, although the preexposure to X-rays was associated with a faster recovery of the mitotic index. It is apparent from plots of the numbers of aberrations observed at the various fixation times against the mitotic indexes (Fig. 3) that the reduction in radon-induced aberrations found when cells are preexposed to 2 cGy of X-rays is independent of the mitotic index, i.e., the speed of cell cycling, and thus is not related to differences in stage sensitivity of the cells sampled.

Because X-rays induce aberrations randomly within cells, the number of cells with 0, 1, 2, 3, etc., aberrations conforms to the Poisson distribution. With α particles from radon, however, the distribution of cells containing 0, 1, 2, 3, etc., deletions does not follow the Poisson distribution

Table 1. Chromatid and isochromatid aberrations induced in human lymphocytes by α particles from radon after a low dose (2 cGy) of X-rays.[a]

Treatment	Fixation time, hr	No. of aberrations Observed	Expected[b]	t
None		4		
X-rays, 2 cGy		10		
Radon, 16.4 cGy	8	138		
X-rays + radon, 15.3 cGy	8	72	138.8	4.60
Radon, 16.4 cGy	11	263		
X-rays + radon, 15.3 cGy	11	182	255.4	3.51
Radon, 16.4 cGy	14	445		
X-rays + radon, 15.3 cGy	14	218	425.2	8.17

[a]Four hundred cells per point were scored. Data from Wolff et al. (*14*).
[b]Expected values are based on the yield from 15.3 cGy of α particles being 0.933 × the induced yield from 16.4 cGy. To this was added the induced yield from 2 cGy of X-rays plus the background value.

Table 2. Mitotic indexes in human lymphocytes at various times after exposure to α particles (16.4 cGy) from radon or to X-rays (2 cGy) plus α particles (15.3 cGy) (*14*).

Fixation time after radon exposure, hr	No. of mitotic cells/No. of cells scored: Control	X-rays	Radon alone	X-rays + radon
8	80/1000 (80)[a]	76/1000 (76)	72/3000 (24)	83/3000 (27.7)
11	ND	ND	108/3000 (36)	131/3000 (43.7)
14	156/2000 (78)	152/2000 (76)	131/3000 (43.7)	149/3000 (49.7)

ND, not determined.
[a]Numbers in parentheses are the numbers of mitotic cells per 1000 cells.

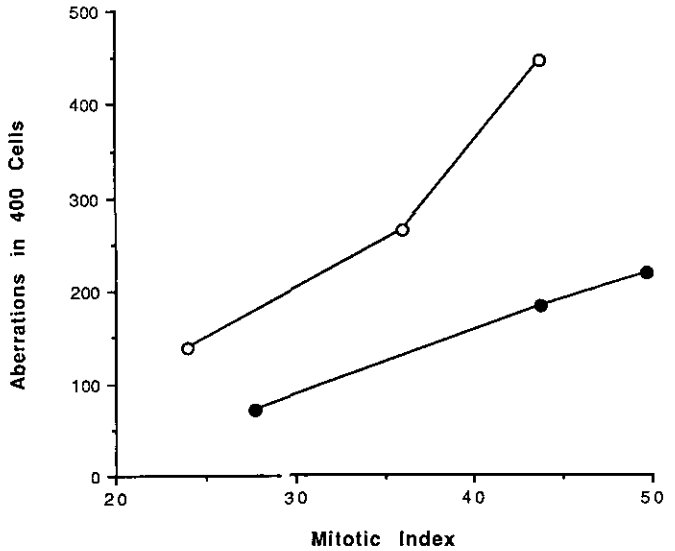

FIGURE 3. Decrease in yields of radon-induced chromatid deletions found in lymphocytes preexposed to 2 cGy of X-rays: independence of the mitotic index. (○) Cells exposed to radon alone; (●) cells exposed to X-rays plus radon. Data from Wolff et al. (*14*).

but is overdispersed, with too many cells containing multiple aberrations and too few containing only one or two aberrations. The distribution obtained is also characterized by an excess of cells without any aberrations. In Table 3, the results of a typical experiment (the 14-hr point in Table 1) illustrate the type of deviation from the Poisson distribution that was found. Such overdispersion has also been noted in studies of full chromosome aberrations induced by α particles in G_1 cells (*17*). When the distribution of aberrations within only those cells that contained aberrations was tested with a conditional, or zero-truncated, Poisson analysis, the overdispersion decreased or even disappeared. At the same time these experiments were carried out, a positive control experiment was performed to see if the usual adaptation was indeed occurring in the cells. Whole blood from the same donor was preexposed to 2 cGy of X-rays and subsequently exposed to 150 cGy of X-rays (data not shown). This experiment showed that, in the blood of donors used for the radon studies, low doses of X-rays led to the expected decrease in the yield of aberrations by subsequent high doses of X-rays.

Discussion

The recent recognition that some homes have high concentrations of radon has focused concern on the potential lung cancer risk associated with indoor exposure to environmental radon. In addressing the risks associated with radon exposures, it is necessary to understand the mechanisms by which radon exposures lead to genetic damage and the exposed cells' response to such damage, including mechanisms for repair of radon-induced chromosomal damage. An important consideration in these studies is the potential difference in the cells' response to high-LET α particles compared with less densely ionizing forms of radiation. The results of the present study indicate that several aspects of the induction of chromatid breaks by α particles from radon are different from those caused by sparsely ionizing X-rays. For instance, with X-rays, cells fixed very shortly after exposure, i.e, cells that are in late G_2 at the time of irradiation, are highly sensitive to radiation. With successive fixations, however, cells in early G_2 and late S are less sensitive. Furthermore, there is a period of relatively uniform sensitivity in cells from these parts of the cell cycle, so that the yield of aberrations remains relatively steady when cells are fixed 6–11 hr after exposure to X-radiation (*1*). With α particles, however, the yield of aberrations increases continuously as washed cells are held after exposure. The lack of uniformity of sensitivity to α particles, which do not hit a sizable fraction of the cells, and the fact that those cells that are hit have an increased chance of excessive damage and mitotic delay probably account for the observed increase in aberration yield with successive fixation times. The results further illustrate the difficulty in estimating the RBE of α particles in the induction of chromosomal aberrations because the yield of aberrations, and hence the RBE, is not

Table 3. Distribution of chromatid deletions in human lymphocytes exposed to either 16.4 cGy of radon alone or 2 cGy of X-rays and 15.3 cGy of radon.[a]

Treatment		Total no. of cells	No. of cells with indicated number of deletions						Total no. of deletions	Mean	χ^2	d-f	p
			0	1	2	3	4	≥5					
Radon	Observed	400	170	110	65	33	11	11	445	1.1125			
	Expected[b] (Poisson)		131.49	146.29	81.37	30.18	8.39	2.28			35.87	3	<0.0001
	Expected (truncated Poisson)	295.6	65.61	98.77	74.34	37.30	14.04	5.57		1.5053	8.91	3	0.03
X-rays + radon	Observed	400	262	82	41	9	4	2	218	0.545			
	Expected (Poisson)		231.94	126.41	34.45	6.26	0.85	0.04			29.15	2	<0.0001
	Expected (truncated Poisson)	218.8	80.75	80.48	40.10	13.32	3.32	0.79		0.997	0.387	1	> 0.5

[a]The cells were fixed 14 hr after radon treatment. Data from Wolff et al. (*14*).
[b]In calculating χ^2, expected values <5 were combined with previous values so that no expected value was <5.

constant but depends on the time after irradiation that the cells are harvested.

Although the study of the adaptive response induced by exposure to low doses of X-rays is more complicated when cells are challenged with high doses of densely ionizing α particles from radon than it is when they are challenged with sparsely ionizing X-rays, in the present study multiple fixation times were used to rule out the possibility that the effects were the result of sampling cells with differential sensitivity to α particles. At all harvest times the yields of radon-induced deletions were reduced in the cultures pretreated with X-rays and, furthermore, were independent of the mitotic indexes.

Although chromosomal breaks induced by high-LET radiations have been thought to be less reparable than those produced by more sparsely ionizing radiations (*13*), the present experiments show that radon-induced chromosomal breaks are subject to repair by the X-ray-inducible adaptive response. Because the molecular lesions responsible for chromosomal breakage are DNA double-strand breaks (*18–22*), the induced repair mechanism must affect their rejoining. The present results further enlarge the spectrum of mutagenic agents whose effects are modified by the putative repair mechanism. These agents include alkylating agents (e.g., MNNG [*N*-methyl-*N'*-nitro-*N*-nitrosoquanidine]), crosslinking agents (mitomycin C) and radiomimetic chemicals (bleomycin) (*23,24*). Furthermore, the observation of an adaptive response to radiation *in vivo* in multiple types of somatic cells as well as germ cells (*8*) indicates the potential for modification of radon-induced chromosomal damage in environmentally exposed populations.

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