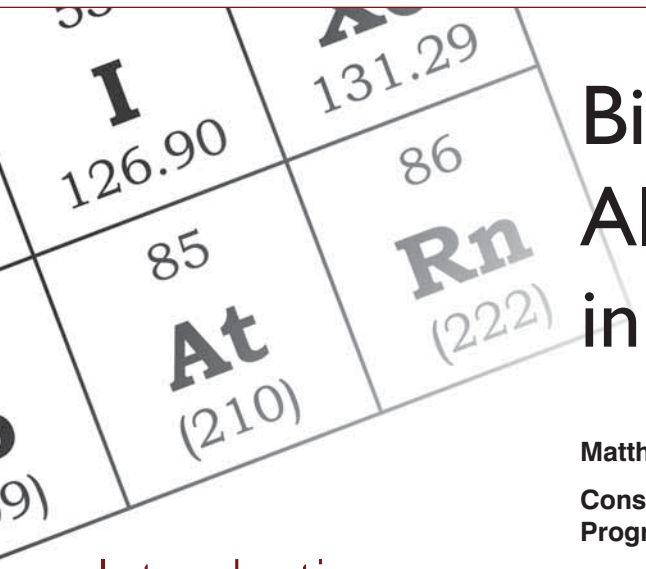

Biological Effects of Alpha Particle Exposure in Human Monocytic Cells

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Introduction

Naturally occurring α(alpha)-radiation is ubiquitous in the environment, its primary source being radon gas. ^{222}Radon is a decay product of ^{226}Radium and ultimately of ^{238}Uranium, both of which are found in soil and rocks and can accumulate in enclosed areas and underground mines. Epidemiological studies conducted among uranium mine workers and using animals have found a strong positive correlation between exposure to radon and an increase in the development of lung cancer (Darby et al., 2005; Kennedy, Gray, Denman, & Phillips, 2002; Samet et al., 1991; Stather, 2004). These studies have triggered considerable concern about similar effects on the general population (Neuberger & Gesell, 2002) and, over the past decade, have prompted several investigations into the effects of residential radon exposure and the risk of lung cancer. The results of these studies have yielded mixed data, with some studies suggesting a positive association between radon levels and lung cancer, and others showing equivocal or negative results (Samet & Eradze, 2000). More recently, combined analysis of data from residential radon case-control studies have shown a measurable risk of lung cancer at radon levels as low as 100 Bq/m^3 (Darby, Hill, & European Collaborative Group on Residential Radon and Lung Cancer, 2003; Krewski et al., 2005).

Radon is an inert, colourless radioactive gas that is derived from the decay of uranium through thorium and radium and continues to decay to radioactive bismuth and polonium through α-particle decay (National Research Council [NRC], 1999). Radon has a half-life of 3.8 days, its concentration in the atmosphere varying, depending on the place, time, and meteorological conditions. It enters buildings from different sources, includ-

Résumé

Des preuves récentes d'un grand nombre d'études en épidémiologie effectuées sur des travailleurs en exploitation minière d'uranium ont démontré que les particules α (alpha) produites par la progéniture de radon provoquent la carcinogenèse des poumons. Pour mieux comprendre le mécanisme moléculaire provoquant ces effets indésirables, des expériences ont été conçues pour étudier la provocation et la réparation des cassures double brin (CSB) et le dégagement de chimiokines pro-inflammatoires de cellules de sang périphérique exposées aux rayonnements α. Les cellules humaines monocytoïdes de sang périphérique (THP-1) ont subi des rayonnements sur six disques électroplaqués de ^{241}Am, dont l'activité s'élevait en moyenne à 68 kBq. Des cultures cellulaires ont été exposées à des rayonnements α en doses de 0 à 2.14 Gy (débit de dose = 0,85 Gy/h). Des cultures alpha-exposées et non exposées ont été cultivées, et les granules de cellules ont été analysées pour déterminer les diverses extrémités. La détection des cassures de brin d'ADN a été évaluée par essai de la comète alcalin. Les surnageants de la culture cellulaire ont été évalués pour déterminer la présence d'une série de chimiokines humains (RANTES, CXC-IP10, IL-8, CXC9/MIG et CC12/MCP-1) par cytométrie en flux à l'aide de microbilles ; le dommage et la réparation subséquente de l'ADN ont été évalués en examinant la formation de γ-H2AX aux sites de CSB d'ADN. Les cellules irradiées avec des particules α variant entre 0,27 et 2.14 Gy démontraient des augmentations statistiquement importantes, proportionnelles à la dose administrée ($p < 0,05$) en formation γ-H2AX, où la dose la plus élevée de provoquait une augmentation presque décuplante chez γ-H2AX. L'essai de la comète n'a révélé aucun dommage notable à l'ADN suite au rayonnement des cellules avec des doses peu élevées (de 0,25, de 0,50 et de 0,75 Gy) relatives au contrôle des cellules, possiblement en raison de la provocation des mécanismes de réparation d'ADN. La détection de sécrétions de chimiokines proinflammatoires dans le médium de culture cellulaire démontre des effets dose-réponse linéaires avec des augmentations statistiquement importantes observées suite à la dose la plus élevée d'exposition aux chimiokines IL-8, RANTES et CXC-IP10. Ces résultats indiquent que le rayonnement a provoque des effets biologiques qui pourraient être compensés en partie par l'activation des processus de réparation cellulaire.

ing soil, rocks, water supplies, natural gas, and building materials. Radon particles decay to a series of solid progeny, which then form into small molecular clusters that can attach to aerosols in the atmosphere. When inhaled, these progeny can settle in lung tissue, whereas the radon gas is mostly exhaled. Inhaled radon and its progeny are absorbed by cells into the bronchial airways and deposited in the lung epithelium. Furthermore, their small size and fat solubility allows them to enter the bloodstream and become deposited in the body (Bowie & Bowie, 1991).
The majority of the energy deposited in biological systems is from the emitted α-particles, which produce a high density of ionization and deliver a large amount of localized energy—about 10–50 cGy. This level of energy is sufficient to cause permanent genetic damage. At the level of DNA, α-particles cause large clusters of multiple ionizations within the DNA and in adjacent molecules, resulting in severely damaged localized sites (Goodhead, 1994; Goodhead & Nikjoo, 1989). Over the years, a great deal of evidence has emerged about the biological effects on cell and animal models and in humans exposed to α-particles. An earlier study conducted by Lutze, Winegar, Jostes, Cross, and Cleaver (1992) showed that freely replicating episomes in human cells exposed to radon gas underwent mutagenic changes, including large deletions involving many thousands of base pairs. Cytogenetic end points such as micronucleus frequency and chromosome aberration formation were also found to increase after exposure to radon and radon progeny, as compared to γ-exposures in both animals and in vitro studies (Jostes, 1996). Furthermore, aberrations in lymphocyte chromosomes and chromosomal instability have been found in the bone-marrow cells of people exposed to radon (Bowie & Bowie, 1991). More recent studies of human lymphocytes have shown increased complexity of chromosome aberrations with exposure to α-particles (Anderson et al., 2007); some of these studies have examined cytokines and apoptosis associated genes and the potential for inflammatory response from α-particle exposure. Exposures of human lung fibroblast cells to low dose α-particle irradiation (3.6–19 cGy) have resulted in the up-regulation of the interleukin-8 (IL-8) gene and protein expression as determined by northern blotting and by ELISA (enzyme-linked immunosorbent assay), respectively (Narayanan, LaRue, Goodwin, & Lehnert, 1999).

In the present study, human-derived monocytic cells were first exposed to α-particles emitted from ^{241}Am electroplated discs and then analyzed quantitatively for the repair of double strand breaks (DSB) using phosphorylated H2AX foci. DSB formation induces the phosphorylation of the tumour-suppressor protein, histone H2AX, and this phosphorylated form, known as γ-H2AX, forms foci at DSB sites. In addition, the alkaline comet assay was employed to measure the amount of DNA damage in relation to the dose of radiation received. Cell-culture supernatants were also tested for pro-inflammatory chemokine production.

Materials and Methods

Exposure and Harvesting

Human-derived peripheral blood monocytic THP-1 cells, obtained from the American Type Culture Collection (ATCC, Manassas, VA, U.S.), were maintained in a humidified incubator at 37°C, 5% CO2, 95% air in 75 cm^2 tissue-culture flasks (Costar, Cambridge, MA, U.S.). The cells were grown to confluence for 2 to 3 days in RPMI1640 medium, containing 10% fetal bovine serum (FBS) (ATCC). The cells were harvested in 35-mm dishes, consisting of inner and outer plastic sleeves and a pierced cap (Chemplex Industries, Palm City, FL, U.S.). Two Mylar membranes (Chemplex Industries), each 2.5 µm thick, were sandwiched between the two sleeves and stretched tightly across the bottom opening. A total of 8.0 x 10^5 cells/dish were seeded into each dish, with 2 mL of culture media containing 100 units/mL of penicillin and 100 µg/mL of streptomycin. The cells were cultured to about 90% confluency, then exposed to α-particle radiation at doses ranging from 0 (control) to 2.14 Gy, using ^{241}Am electroplated discs with an activity level of 68.0 kBq ± 3% at a dose rate of 0.85 Gy/h. The cells were harvested either immediately after exposure (alkaline comet assay) or 30 minutes post exposure (γ-H2AX). Five independent experiments were conducted for the comet assay and four were conducted for the H2AX assay. Cell viability was assessed from a 30 µL aliquot, both prior to exposure and immediately after exposure, at all doses by the dual-stain viability assay (Strauss, 1991).

Dosimetry

The dosimetry of the exposure system was modelled using the GEANT4 Monte Carlo tool kit. The cells were maintained in tissue-culture flasks with a surface area of 75 cm^2 (T75) and a canted neck with ventilated caps (VWR, Mississauga, ON, Canada). In order to render an environment suitable for α-exposure, the cells were cultured in thin, Mylar-based plastic dishes (MD), which α-particles are able to penetrate. The MD consisted of a three-piece plastic sleeve: inner and outer sleeves, with a pierced cap that fit tightly to the dish (Chemplex Industries, Palm City, FL, U.S.). Two Mylar membranes (Chemplex Industries), each 76.2 mm in diameter and 2.5 µm thick, were placed across the bottom opening of the inner sleeve. The outer sleeve was then slid over the membranes, leaving the membranes sandwiched between the two sleeves and stretched tightly across the bottom opening. The MD pieces were autoclaved in loosely sealed glass jars at 121°C for 25 minutes and were then left to cool to room temperature before the dish was put together. The energy of the α-particles directly incident on the nuclei of the cells was a distribution of energies with a mean of 3.33 ± 0.11 MeV and the average linear energy transfer was 154.8 ± 4.7 KeV/um. These values being calculated from the geometry discussed above.

H2AX Phosphorylation Assay

H2AX phosphorylation was assessed using flow cytometry, according to the following protocol. Thirty minutes following exposure, cell suspensions (500,000 per sample) were washed and fixed with 4% formaldehyde (Fisher Scientific, Hampton, NH, U.S.) and incubated for 15 minutes on ice. The cells were then washed and re-suspended in 1 mL cold (-40°C), 70% methanol (Fisher Scientific) and stored at -40°C for at least overnight and up to two weeks. Subsequently, 1 mL of cold TBS (Triphosphate Buffered Saline, 0.0154 M Trizma Hydrochloride [Sigma-Aldrich Canada, Oakville, ON], 0.5 M NaCl [Fisher Scientific], pH 7.4) was added to each sample, mixed well, centrifuged (8 min, 400 x g, 4°C), and re-suspended in 1 mL of cold TBS Serum Triton (TST, 96% TBS, 4% FBS [Sigma-Aldrich], 0.1% Triton X-100 [Sigma-Aldrich]). The samples were incubated on ice for 10 minutes, centrifuged (5 min, 400 x g, room temperature), and re-suspended in 200 µL of anti-γ-H2AX-FITC (Fluorescein isothiocyanate) antibody (Upstate Biotechnology, Waltham, MA, U.S.) that was diluted 1:500 in TST. After a 2-hour incubation period on a shaker platform at room temperature, 1 mL of TBS with 2% FBS was added. The samples were then centrifuged (5 min, 400 x g, 4°C) and re-suspended in 300–400 µL TBS with 2% FBS. Just prior to analysis by flow cytometry, 2 µL of 1 mg/mL propidium iodide (PI) was added to each sample.

Flow Cytometry Analysis

For flow cytometry analysis, data acquisition was set to analyze 20,000 cells on forward scatter (FSC) versus side scatter (SSC). The γ-H2AX response was measured by assessing the increased level of intracellular fluorescence characterized in the cells, as determined by the X-geometric mean (channel number) of the γ-H2AX positive cells. The cell-cycle distribution was assessed by examining the distribution of the area of the PI signal. All samples were analyzed on a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, U.S.).

Alkaline Comet Assay

The modified alkaline comet assay was performed as described previously (McNamee, McLean, Ferrarotto, & Bellier, 2000). The cells were exposed to α-radiation, and immediately after exposure, 50,000 cells were mixed with agarose (1%) in PBS at 37°C in a 1:10 dilution. The gels were cast on Gelbond strips (Mandel Scientific, Canada), using gel-casting chambers, and then placed into a lysis buffer (2.5 M NaCl, 0.1 M tetra-sodium EDTA, 10 mM Tris-base, and 1% (v/v) Triton X-100, pH 10.0), after which the gels were rinsed with distilled water, incubated in an alkaline electrophoresis buffer for 30 minutes at room temperature, and electrophoresed at 20 V for 20 minutes. They were subsequently rinsed with distilled water and placed in 1 M ammonium acetate for 30 minutes, then soaked in 100% ethanol for 2 hours, dried overnight, and stained with SYBR Gold (1/10,000 dilution of stock from Molecular Probes, Eugene, OR, U.S.) for 45 minutes. Comets were visualized at 220X magnification, and DNA damage was quantified, using the tail-moment parameter (i.e., the distance between the barycenter of the head and the tail of the comet multiplied by the percentage of DNA within the tail of the comet), ratio (% of DNA in the tail), comet length, and tail length. A minimum of 50 cell comets were analyzed for each sample, using ALKOMET Version 3.1 image analysis software.

Cytokine Analysis

Twenty-four hours post exposure, cell-culture supernatants (2 mL) from each treatment group were lyophilized overnight and then reconstituted in 300 µL of culture media. The samples were then centrifuged at 500 x g for 5 minutes to remove any sediment. Following that, the cytokine concentrations were quantified, using the BD Human Chemokine Kit Cytometric Bead Array (CBA) assay (BD Biosciences), and analyzed on a BD FacsCalibur flow cytometer (BD Biosciences).

Statistical Analysis

Statistical differences ($p \leq 0.05$) between treatment groups and control groups were determined by a repeated-measures design one-way ANOVA with Dunnett's multiple comparisons post hoc testing. Comet data were presented as mean ± SEM of five independent experiments, γ-H2AX data as mean ± SEM of four independent experiments, and cytokine data as mean ± SEM of six independent experiments.

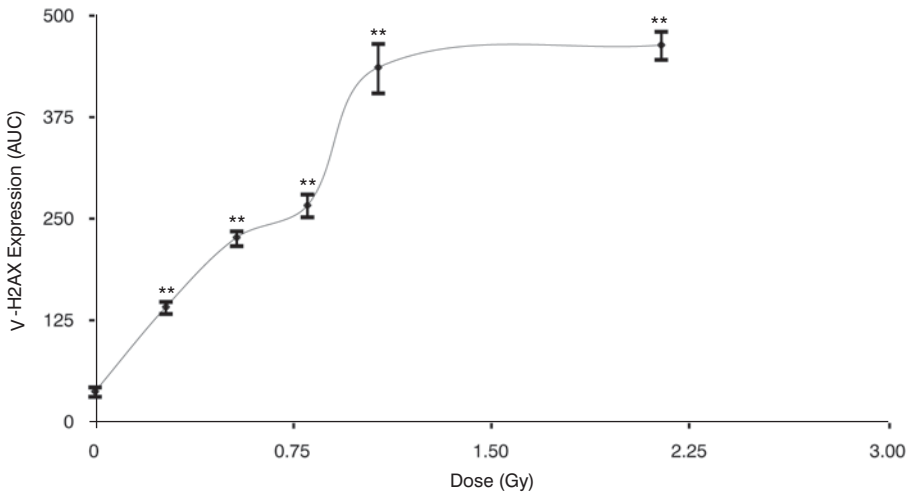


Figure 1: γ-H2AX expression at various doses. Data are presented as means ± SEM with n = 4 biological replicates. ** represents $p \leq 0.01$ statistically significant difference from the control. A linear trend was observed up to 0.50 Gy ($R^2 = 0.96$; line not shown).

Results

Cellular Viability

To assess the overall integrity of the cells, cell viability and cell number were measured following all exposure conditions. The results indicated that α-radiation at all doses and time points tested did not cause any significant effects on cellular viability. The cells remained 95–99% viable, and no statistically significant decreases in cell number were observed following α-particle irradiation (data not shown).

H2AX Phosphorylation

An immunocytochemical assay using antibodies capable of recognizing histone H2AX phosphorylated at serine 139 (γ-H2AX) was employed to assess DNA damage and repair following the exposure of monocytic cells to α-radiation. To determine the optimal time point for γ-H2AX expression, a time-course experiment was conducted up to 120 minutes post-irradiation with 1 Gy of α-radiation; γ-H2AX induction was found to peak at 30 minutes (data not shown). Therefore, for all experiments, γ-H2AX was assessed 30 minutes post-irradiation. To ensure that any increases in γ-H2AX in our treatment groups were not related to increases in DNA content as a result of the cell-cycle phase, the DNA content of the cell pellets was measured, and it was found to remain constant for all sample treatment groups. Monocytic cells exposed to α-radiation at various doses showed a dose-responsive increase (Figure 1) up to 1.07 Gy of irradiation, at which point expression reached a plateau. Statistically significant responses were obtained at all doses tested ($p \leq 0.05$) relative to the unirradiated control treatment group. At the highest dose (2.14 Gy), approximate10-fold increase in γ-H2AX was observed relative to the control sample.

Table 1: DNA damage parameters for comet assay were determined following irradiation of monocytic cells to gamma (^{137}Cs) and α-radiation (^{241}Am). Data are presented as means ± SEM from n = 5 biological replicates for α-exposures and n = 3 biological replicates for γ-exposures. * represents $p < 0.01$ statistical difference compared to the relative control.

Radiation Type	Dose (Gy)	Ratio	Moment	Comet Length	Tail Length
α	0	0.09 ± 0.03	1.31 ± 0.50	47.9 ± 12.2	24.1 ± 9.9
	0.25	0.09 ± 0.02	1.13 ± 0.30	48.0 ± 9.5	24.5 ± 7.6
	0.50	0.10 ± 0.03	1.45 ± 0.70	55.1 ± 7.5	30.3 ± 5.8
	0.75	0.11 ± 0.03	1.53 ± 0.50	57.0 ± 11.2	32.1 ± 9.1
γ	0	0.20 ± 0.05	2.10 ± 0.40	42.0 ± 2.6	21.4 ± 3.1
	4	0.30 ± 0.06	5.14 ± 1.90*	73.8 ± 5.5*	47.7 ± 5.6*
	8	0.45 ± 0.01*	10.7 ± 0.7*	75.3 ± 1.7*	49.7 ± 1.8*

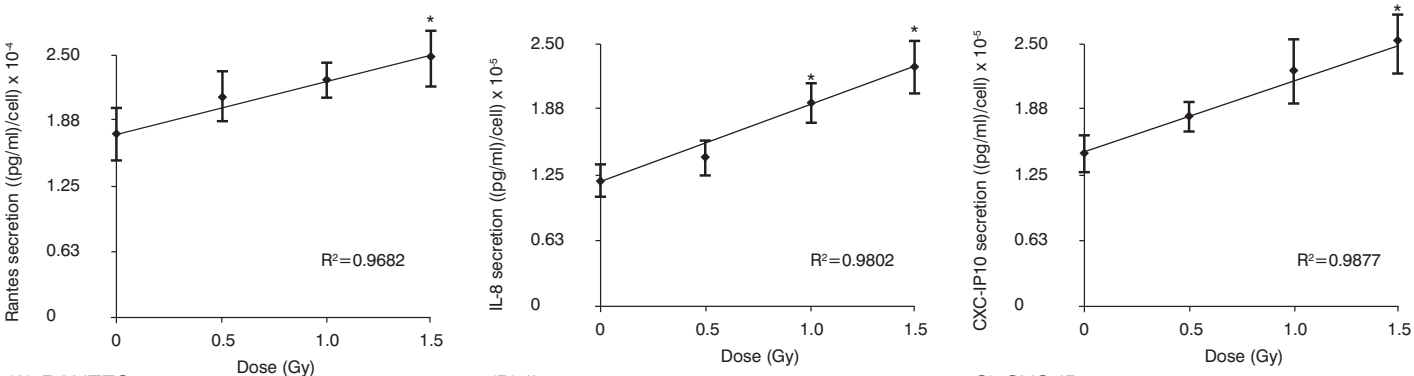
Alkaline Comet Assay

The ability of α-particle exposure to elicit DNA strand breaks was assessed in human monocytic cells using the alkaline comet assay. DNA damage was assessed in the cell cultures immediately after exposure to between 0 and 0.75 Gy α-particle radiation. DNA damage was quantified in a minimum of 50 cells, using tail ratio, tail moment, tail length, and comet length as measures of DNA damage. Alpha-irradiation did not result in statistically significant DNA damage by any of the four parameters at doses of 0.25, 0.50, and 0.75 Gy (Table 1). However, a positive control that comprised cells exposed to 4 and 8 Gy of γ-radiation did produce statistically significant increases in single and DSBs ($p \leq 0.05$), compared to the control.

Cytokine Production

The protein levels of RANTES, CXC-IP10, IL-8, CXC9/MIG, and CC12/MCP-1 were monitored in cell-culture supernatants for all samples, using a multiplex cytometric bead array assay, and were quantified by flow cytometry. Of the four chemokines tested, only RANTES, CXC-IP10, and IL-8 could be detected in the culture media of THP-1 cells (Figure 2); CC12/MCP-1 and CXC9/MIG were below the threshold of detection. The secretion of RANTES into the cell-culture media was found to be 10-fold higher in comparison with

Figure 2: Secretion levels following exposure to various doses of α-radiation. Data are presented as means ± SEM with n = 6 biological replicates. All graphs show linear trends with $R^2 \geq 0.95$.



(A) RANTES (B) IL-8 C) CXC-IP10

* represents a $p \leq 0.05$ statistical difference compared to the control.

CXC-IP10 and IL-8. Overall, a linear dose-response effect was observed, with statistically significant increases in chemokines observed at the highest dose tested (1.5 Gy), with the exception of IL-8—its concentration was found to have increased in cell-culture supernatants following 1.0 Gy of α-radiation.

Discussion

Recent animal-based and epidemiological studies have shown radon inhalation as the second-highest cause of lung cancer after smoking (Frumkin & Samet, 2001). Inhaled radon and its progeny are absorbed by cells into the bronchial airways and deposited in the lung epithelium, where they easily enter the bloodstream to induce toxic effects. Since radon-induced carcinogenesis has been studied mainly through epidemiological investigations, well-controlled in vitro and in vivo experiments are needed to provide further insights into the biological mechanisms underlying radon exposure-induced adverse effects.

In this study, blood monocytic cells were examined, based on the critical role they play in the body's defence against foreign substances. Various biological end points were examined, including DNA damage induction and repair, cellular viability, and chemokine secretion. Homeostatic imbalances in any one of these end points could result in a compromised cellular system, leading to carcinogenesis. Because DNA is well known as a critical target for the biological effects of radiation, DNA damage was examined, using two methods: the highly sensitive γ-H2AX assay and the alkaline comet assay.

H2AX is a histone variant that is ubiquitously expressed throughout the chromatid structure. Numerous studies have shown that DNA damage that results in the induction of DSB induces the phosphoryalation of H2AX at serine 139. The phosphorylated form of this histone, known as γ-H2AX, can be detected with an immunocytochemical assay that utilizes antibodies that recognize γ-H2AX. This type of assay has become the standard for both the detection of DNA damage and its repair (Takahashi & Ohnishi, 2005). In this study, the optimal time point of γ-H2AX expression was found to be 30 minutes post-irradiation. Monocytic cells dosed with α-radiation ranging from 0–2.14 Gy showed a linear response in the induction of γ-H2AX up to 1.07 Gy (0.50 to 1.07), after which the expression of γ-H2AX reached a plateau, conceivably due to the low dose rate of α-exposure. For the highest dose, the time course of exposure was approximately 2.4 hours; therefore, the amount of γ-H2AX observed over the 2.4-hour mark depended upon the rate of phosphorylation of H2AX and the rate of dephosphorylation as repair progressed. This study, and others, have shown that γ-H2AX appears within minutes of the induction of DSB, increasing with time up to 30 minutes and then decreasing as the DSB are repaired (Antonelli et al., 2005; Pilch et al., 2003; Rogakou, Boon, Redon, & Bonner, 1999; Rogakou, Pilch, Orr, Ivanova, & Bonner, 1998). It follows that the amount of γ-H2AX observed in cell cultures after a longer exposure at a low dose rate would be less than the amount observed after acute exposures of the same dose. The cells will reach steady-state equilibrium between the amount of DNA being damaged and the amount of DNA being repaired. Only one other study has examined the formation and repair of DSB after exposure to α-radiation. That study used γ-H2AX as an indicator of damage in Chinese hamster cells, and the researchers were able to show a linear dose-response curve up to 1 Gy of α-radiation, followed by a slight decrease but not a plateau in γ-H2AX induction at the higher dose (Leatherbarrow, Harper, Cucinotta, & O'Neill, 2006). This discrepancy was likely the result of the dose rate used for exposure, as their study employed an α-source with a dose rate of 1–2 Gy/min.

The alkaline comet assay was employed to assess DNA damage in the current study because, under alkaline conditions, all single-strand DNA breaks can be detected regardless of whether they result from DNA double-strand lesions, original single-strand breaks, or abasic sites. Monocytic cells exposed to α-radiation (dose rate 0.85 Gy/h) at a dose of 0.25, 0.50, and 0.75 Gy did not show any significant increase in DNA damage following irradiation. However, our γ-irradiated control (dose rate 1 Gy/min) showed significant DNA damage at 4 and 8 Gy of irradiation. These results are similar to a study conducted by Rössler et al. (2006), in which no significant effects on DNA damage were observed using the alkaline comet assay in confluent human gastric cancer cells exposed to ^{241}Am (α-emitter); DNA damage was only detectable at doses of >2 Gy. The lack of DNA damage detected by the comet assay in this study was probably due to the low dose rate for α-exposure (0.85 Gy/h). It has been shown in C3H10T1/2 cells that the repair kinetics response in cells is a biphasic response. The fast component of repair occurs in the first two hours after irradiation; a slow component repairs the residual breaks within 24 hours (Banáth, Fushiki, & Olive, 1998; Nocentini, 1999). At a low dose rate (0.85 Gy/h), most of the DNA damage that has occurred will be repaired by the end of the exposure, a finding that is supported by the γ-H2AX data that show a strong induction of DNA repair mechanisms at low doses of radiation.

Chemokines are a family of low molecular weight, pro-inflammatory cytokines, which bind to G-protein coupled receptors. They function primarily in chemoattraction and activation of specific leucocytes in various immuno-inflammatory responses. Recent studies have shown them to be key components in cancer, involved in the neoplastic transformation of cells (Arya & Patel, 2003). In our study, we evaluated the production of a number of chemokines after the exposure of cells to α-radiation. Of the five chemokines tested, only three responded to the α-radiation. Although the three responsive chemokines (RANTES, IL-8, and IP-10) showed a linear dose-response trend, significant changes were only observed at the highest dose tested (1.5 Gy), with the exception of IL-8, which also showed significance at the medium dose. Interleukin-8 is a chemokine that is known to be up-regulated by a stressor response, and it is recognized as a potent chemoattractant and activator of neutrophils, lymphocytes, and basophils. Researchers have found that activated leukocytes (c to k) can produce and release large amounts of reactive oxygen species (ROS) and toxic granules (i.e., myeloperoxidases), leading to excessive inflammation (Caricchio, McPhie, & Cohen, 2003). Narayanan et al. (1999) showed that α-exposed human normal fibroblasts induced increases in production of IL-8 in parallel with elevated production of ROS. Such production of IL-8 induced by α-particles may contribute to an inflammatory response in the lower respiratory tract.

Summary

It has been shown here that α-radiation causes significant biological effects in terms of DNA damage and chemokine release. However, it is evident that, at a low dose rate of α-exposure such as would occur in a physiological setting, most of the DNA damage is repaired by the recruitment of repair proteins. This is indicated by the induction of γ-H2AX at low doses and a corresponding lack of DNA damage in the comet assay. Although significant differences were observed in chemokine secretion, the in vivo biological effects of these differences are unclear. Although chemokines have been shown to be beneficial, inducing proliferation, differentiation, and cell death (Yarilin & Belyakov, 2004), these chemokines can also strongly activate inflammatory responses and cell death in various tissues, including the lung. Thus, with both beneficial and negative consequences, in vivo models are warranted to better understand the risks associated with low dose rate α-particle irradiation, such as occurs through radon gas inhalation. ■

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