Mutation Research 757 (2013) 79–82

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Synergistic effect of radon in blood cells of smokers – An *in vitro* study

C. Meenakshi, Mary N. Mohankumar*

Radiological Safety Division, Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam 603102, Tamilnadu, India

ARTICLE INFO

Article history:
Received 27 December 2012
Received in revised form 13 June 2013
Accepted 21 June 2013
Available online 9 July 2013

Keywords:
DNA damage
Radon
Smokers
Synergistic effect
Chromosome aberrations

ABSTRACT

Epidemiological studies indicate that the risk of lung cancer among smokers increases with exposure to residential radon. The present study aimed to investigate the synergetic effect between smoking and radon. Blood samples from smokers and non-smokers were exposed to different concentrations of radon ranging from 0 to 189 MBq/m^3 corresponding to doses ranging from 0.2 to 15.2 mGy. Chromosome aberrations in first division metaphase preparations were scored. The frequency of dicentrics in radon-exposed smoker cells was found to be higher than non-smokers by factor of 3.8. The present study is the first of its kind to investigate the interaction of radon and smoking sans confounding factors, as smoker cells were exposed *in vitro* to radon.

1. Introduction

Radon is rated as a group one carcinogen by the International Agency for Research on Cancer [1]. Radon is emitted from uranium, a naturally occurring mineral in rocks and soil; thus is present virtually everywhere on the earth.

According to the BEIR IV report of the National Academies of Sciences, for men exposed to radon at work, smokers are 10 times likely to get lung cancer compared to non-smokers. Besides, smokers dwelling in homes with high radon levels (>200 Bq/m^3) are estimated to be 6–8 times at a higher risk compared to non-smokers [2]. Nevertheless, studies on the effect of elevated radon levels in homes remain inconclusive. Urban areas tend to have lower radon concentrations than rural, as the underlying rock is usually sedimentary added to the probability that more people live upstairs in apartments. Nevertheless, urban areas also usually have a higher prevalence of smoking. If however, people with higher residential radon concentrations tended to smoke less, assessment of the magnitude of the risk associated with radon becomes difficult. Other confounding factors include years since stopping smoking and amount smoked for ex-smokers.

Studies show mounting evidence for a multiplicative synergistic effect between radon and smoking and estimates indicate that the risk of lung cancer may be multiplied up to 18-fold when combined with cigarette smoking [3]. Other reports estimate relative risks to be substantially lower those derived from the multiplicative model, but somewhat higher than those from the additive model [4,5]. However, these estimates are solely based on epidemiological studies, mostly on miners wherein the association between radon and smoking alone has not been well established as it is unclear if this may be due to other factors such as mineral dusts present in the mining environment. Further, a recent nested case–control study fails to reveal enhanced risks [6]. Thus the combined effect of smoking and radon still remains controversial in risk assessment.

Cytogenetic biomarkers have proved to be excellent methods to detect and estimate radon- and progeny-induced DNA damage in miner cells [7–10]. Among the various cytogenetic biomarkers structural chromosomal aberrations are still regarded as the most sensitive, exhibiting a dose response and thus widely used in biological dosimetry.

As epidemiological studies are often confounded by lifestyle and demographic parameters, *in vitro* studies have the advantage that such confounding factors such as smoking and co-exposure to known or suspected carcinogens (diesel exhaust, arsenic, and silica dust) could be eliminated.

The aim of the present study was to assess chromosome damage induced by radon and smoking and to estimate the extent of synergy in their capacities as DNA damaging agents.

2. Materials and methods

2.1. Study group

The study was conducted among cigarette smokers and non-smokers. There were 26 non-smokers (only males) aged between 22 and 56 years and 28 smokers (only males) aged between 21 and 54 years who smoked about 6–18 cigarettes per day for periods ranging between 7 and 30 years. The mean age of the non-smokers group and smokers group were 36.1 years and 35.5 years, respectively. Reports

* Corresponding author. Tel.: +91 44 27481376; fax: +91 44 27480228.
E-mail addresses: marynmk@gmail.com, marynmk@rediffmail.com (M.N. Mohankumar).

1383-5718/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mrgentox.2013.06.018

[11,12] suggest that smokers enrolled in the present study could be classified as "moderate smokers".

Blood samples were collected using heparinised vacuette tubes (Greiner Labortechnik, Austria) by vein-puncture, after prior consent, from apparently healthy smoking and non-smoking individuals working at the Indira Gandhi Centre for Atomic Research (IGCAR), Kalpakkam. All the subjects were informed about the purpose and procedure of the study and they signed an informed consent as per regulations of the departmental ethics committee. They were also asked to fill in a detailed questionnaire to obtain information regarding occupational exposure to radiation, medical exposures (such as X-ray/CT scan), personal habits, alcohol consumption, frequency and period of smoking (pack years).

All the subjects belonged to the same ethnic group and did not differ significantly in their dietary habits. They were neither engaged in radiation work nor were they exposed to any diagnostic X-rays during the past 5 years. Among the non-smokers, none of the subjects had ever been a smoker. Both smokers and non-smokers were inhabitants of Kalpakkam for more than 10 years.

Pack-years, an indicator of cumulative smoking dose is defined as the number of packs of cigarettes smoked per day multiplied by the duration of smoking in years.

2.2. Irradiation procedure

A novel radon irradiation assembly developed and tested at the Radiological Safety Division of IGCAR was used for irradiation of blood and has been described in detail elsewhere [13]. Briefly, radon–air mixture produced from a sealed source (Pylon-Model RN-1025, Canada) containing dry radium powder was used to irradiate blood samples taken in aseptic glass bottles of 100 ml capacity using a three-way cock connected to a 60 ml syringe. Using this assembly it was possible to deliver an exact volume of radon to blood and another exact volume to an evacuated Lucas cell for measuring radon concentration using an alpha counter. By varying the volume of radon gas taken in the syringe for each experiment different doses could be delivered.

About 10 ml of freshly collected blood samples from each non-smoker were aliquoted into two airtight glass bottles of 100 ml capacity. One served as control and the other was exposed to one dose of radon. Thus, samples collected from the 26 non-smokers were exposed to radon doses ranging between 0.4 and 15.2 mGy. Blood samples from 28 smokers were exposed likewise to doses ranging between 0.2 and 11.2 mGy. Doses were estimated using a Lucas cell and an alpha counter. Immediately after irradiation, bottles containing blood samples were placed on a rocker platform inside a 37 °C incubator for 3 h for uniform irradiation, after which they were subjected to the chromosome aberrations assay.

2.3. Chromosome aberrations assay

About 1 ml whole blood was added to 9 ml of RPMI 1640 containing 100 U/ml penicillin, 100 μg/ml streptomycin and 1 ml of foetal bovine serum (Himedia, India). Bromodeoxyuridine to a final concentration of 10 μM was added to differentiate first division cells. Cultures were initiated by the addition of 5 μg/ml phytohemagglutinin (Gibco) and incubated for 48 h in 5% CO_2 atmosphere. At the 45th hour, colchicine (Sigma) to a final concentration of 0.04 μg/ml, was added to arrest cells at metaphase. Cultures were harvested at 48 h and subjected to a hypotonic treatment of 0.56% KCl. Cells were washed and suspended in Carnoy's fixative, cast on microscope slides, air-dried, stained with giemsa and scored for aberrations in first division metaphases. All slides were coded and scored without bias. A minimum of 500 cells per individual was scored.

Metaphases were captured using an automated metaphase finder system (Metasystems, Germany). Individual metaphases were carefully analysed for aberrations and noted onto scoring sheets. The following aberrations were scored: dicentric (a chromosome with two centromeres along with its associated fragment), acentric fragments (a pair of broken portions of chromatid arms which may or may not be lying in close vicinity of the original chromosomes), breaks (damage to a chromatid involving a discontinuity of the chromosomes greater than the width of the chromatid), minutes (small dot-like fragments) and centric ring (a ring shaped chromosome resulting from an exchange between two breaks occurring on either side of the centromere).

2.4. Statistical methods

Statistical analyses were performed using the INSTAT software. All data from the assays were tested for normality. Since the test did not show normal distribution, the nonparametric Mann–Whitney test was used. Statistically significant differences were tested at 1% and 5% levels. Correlation was calculated according to Spearman and the level of significance set at 95% ($\alpha = 0.05$).

3. Results

A total of 17,086 cells from 26 non-smokers unexposed to radon, hereafter referred to as control non-smokers and 15,832 cells from the same 26 non-smokers exposed to radon hereafter referred to as non-smokers (exposed) were scored. Likewise 24,205 cells from 28 smokers unexposed to radon, hereafter referred to as control smokers and 14,841 cells from the same smokers exposed to radon hereafter referred to as smokers (exposed) were scored. The aberrations from the unexposed aliquots were subtracted from the exposed to obtain net values.

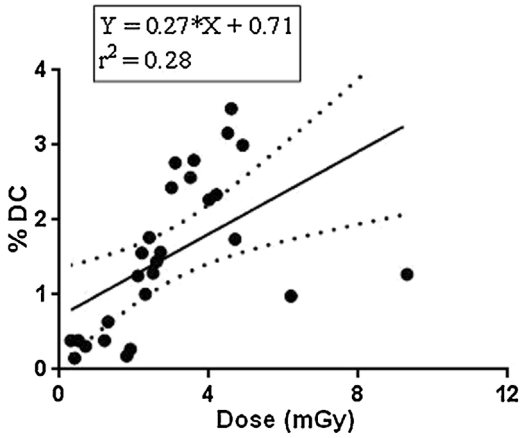

Fig. 1. Variation of dicentric yield with radon dose (smokers). Closed circle indicates each individual's value of dicentric chromosome after exposure to different concentrations of radon gas exposure. Upper and lower dotted lines show upper and lower range of 95% confidence interval, respectively.

The mean percentage of dicentrics, acentric fragments, chromatid breaks and minutes among the control smokers were 0.032 ± 0.07, 0.08 ± 0.14, 0.07 ± 0.12 and 0.068 ± 0.15, respectively. These values in cells of control non-smokers were 0.027 ± 0.05, 0.045 ± 0.10, 0.035 ± 0.11 and 0.029 ± 0.08, respectively (Table 1).

The mean percentages of dicentrics, acentric fragments, chromatid breaks, minutes and centric rings in smokers (exposed) were 1.566 ± 1.02, 1.63 ± 0.87, 1.41 ± 1.04, 0.860 ± 0.72 and 0.045 ± 0.08, respectively and these values in non-smokers (exposed) were 0.663 ± 0.53, 0.926 ± 0.67, 0.827 ± 0.66, 0.633 ± 0.78 and 0.003 ± 0.01, respectively (Table 1). The frequencies of dicentrics and acentric fragments in exposed smokers were very significant compared to exposed non-smokers ($P < 0.005$) whereas the frequency of chromatid breaks was considered to be significant ($P < 0.05$). The frequency of centric ring in exposed smokers was not significant compared to exposed non-smokers (Table 1).

The frequency of dicentrics, acentric fragments, chromatid breaks and minutes in exposed smokers were extremely significant than that of control smokers ($P < 0.0001$). The frequency of dicentrics, acentric fragments, chromatid breaks and minutes of exposed non-smokers were also extremely significant than that of control non-smokers ($P < 0.0001$). While acentric fragments, chromatid breaks and minutes were observed in the controls of smokers and non-smokers, centric rings were not observed in both the control groups whereas the same was observed in the radon exposed groups (smokers & non-smokers).

Figs. 1 and 2 show the dose-responses to the dicentric yields in smokers and non-smokers, respectively. A slope ratio value of 3.8 was deduced, even though the correlations were too weak.

Spearman rank correlation for the frequency of dicentrics with respect to dose was extremely significant ($P < 0.0001$) in both smokers and non-smokers. The frequency of centric ring with respect to dose in smokers was significant ($P < 0.05$). No correlation was observed for non-smokers (data not shown). No correlation was also observed for the frequencies of acentric fragments or chromatid breaks in both non-smokers and smokers (data not shown).

Pack-years, an indicator of cumulative smoking dose, was estimated for all smokers but showed no significance with respect to