Original Manuscript

Assessing the level of chromosome aberrations in peripheral blood lymphocytes in long-term resident children under conditions of high exposure to radon and its decay products

Vladimir G. Druzhinin¹,², Maxim Yu. Sinitsky¹,*, Aleksey V. Larionov¹, Valentin P. Volobaev¹, Varvara I. Minina² and Tatiana A. Golovina¹

¹Department of Genetics, Biology Faculty, Kemerovo State University, Krasnaya St. 6, Kemerovo 650043, Russian Federation and ²Institute of Human Ecology of SB RAS, Leningradsky Ave 10, Kemerovo 650065, Russian Federation

*To whom correspondence should be addressed. Tel: +7 923 601 2486; Fax: +7 3842 583885; Email: sinitsky.maxim@gmail.com

Received 5 December 2014; Revised 11 March 2015; Accepted 11 March 2015.

Abstract

In this study, the frequency and spectrum of chromosomal aberrations were analysed in samples of peripheral blood from 372 (mean age = 12.24 ± 2.60 years old) long-term resident children in a boarding school (Tashhtagol city, Kemerovo Region, Russian Federation) under conditions of high exposure to radon and its decay products. As a control group, we used blood samples from people living in Zarubino village (Kemerovo Region, Russian Federation). We discovered that the average frequencies of single and double fragments, chromosomal exchanges, total number of aberrations, chromatid type, chromosome type and all types of aberrations were significantly increased in the exposed group. This is evidence of considerable genotoxicity to children living under conditions of high exposure to radon compared to children living under ecological conditions without increased radon radiation.

Introduction

Estimating the biomedical long-term effect of small doses of ionising radiation is a complicated issue that affects not only radiobiology but also social and economic spheres. Greater than 60% of the ionising radiation a person receives each year can be caused by natural sources of radiation, and >50% of this radiation can be caused radon and its decay products (1). Therefore, maintaining radon safety is one of the most critical challenges in modern ecology and genetic toxicology.

Radon (²²²Rn) is a naturally radioactive noble gas. It is generated from uranium, a chemical element that is widespread in the earth’s crust. Radon is electrically neutral and is not itself a potential health threat, but its decay daughter products, ²¹⁸Po, ²¹⁶Po, ²¹⁶Pb and ²¹⁸Bi, are electrically charged and can affix themselves to tiny dust or smoke particles in indoor air. These particles can be inhaled into the lung where they may penetrate the epithelial cells that cover the bronchi and alveoli. These short-lived, unstable decay daughter products, particularly ²¹⁸Po and ²¹⁶Po, emit alpha particles that can interact with biological tissues in the lungs and induce DNA damage (2,3). There are many epidemiological studies on the connection between radon influence and some cases of cancer, particularly lung cancer (3–11).

However, it is important to note that current models estimating the risk of radiation-related hazards are based on the analysis of data collected from irradiated miners. Currently, it is not clear whether this risk model can be used for studying the inhabitants of domestic areas under high-radiation conditions (11).

Estimating the effects of radon exposure on the population in the territories of regions with developed mining industries is of particular interest. The Kemerovo Region is such a territory (12). Therefore, a portion of the population living in the coal-mining area may be exposed to a long-term radiation risk from radon and its decay products. In addition to several methods of radiation monitoring, the level and nature of such effects should be determined using biological indication methods. The World Health Organization recommends estimation of the frequency and spectrum of structural chromosome aberrations in cultured lymphocytes for the biological indication of the effects of radiation on human populations (13). The importance of these indicators of genotoxic effects is evidenced by...
the finding that the frequency of chromosome mutations detected in somatic cells is a marker of susceptibility to cancer (14–18).

Materials and Methods

Group description

Blood samples were obtained from 372 (mean age = 12.24 ± 2.58 years old) long-term resident children in a boarding school (Tashtagol city, Kemerovo Region, Russian Federation). This area is characterised as a wood-mountain area with a low level of air pollution from chemical agents, such as polycyclic aromatic hydrocarbons and heavy metal salines, which may induce some cytogenetic abnormalities. We used the bioindicative methods of air and water analyses, including the Ames test to assess the air mutagenic activity and test to induce dominant lethal mutations in Drosophila with water and air samples, to exclude chemical mutagenesis factors.

This region is characterised by the intensive mining of minerals, such as iron ore and coal; therefore, some portions of the territory are contaminated by ash and slag (19). In addition, the measurement of radon volume activity performed in the rooms of the boarding school showed an excess critical concentration (>200 Bq/m³) (Table 1).

The optimal opportunity to estimate the significance of a radiation factor as a potential modifier of the spontaneous level of chromosomal aberrations is achieved by the age requirement during the formation of a study group. From this perspective, the group of children and adolescents may be the most useful, and in this case, the influence of factors, such as bad habits and occupational exposure to industrial hazards, is minimised. In addition, the compact residence of all members of the sample permits the maximum similarity of the studied group for nutrition and living conditions.

In the control group, 186 children (mean age = 14.43 ± 2.58 years old) were included. They live in settlements without increased radon levels (Table 1). The gender and age characteristics are presented in Table 2. Nutrition for the control group has been similar as for the exposed group.

The children receiving medical treatment, as well as having received an X-ray examination 3 months prior to collection of the material, were not included in the study. For each person, informed consent signed by the parents or persons with custody of the minors was obtained. The research has been performed in accordance with the requirements of the Ethics Committee of the Kemerovo State University.

Table 1. The radon volume activity in buildings of the studied settlements

<table>
<thead>
<tr>
<th>Settlement</th>
<th>Date of measurement</th>
<th>Number of measuring points</th>
<th>Average radon volume activity, Bq/m³, mean ± standard error</th>
<th>Limit variation, Bq/m³–Bq/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tashtagol</td>
<td>20.12.2007</td>
<td>11</td>
<td>235 ± 44</td>
<td>68–583</td>
</tr>
<tr>
<td>Tashtagol</td>
<td>06.02.2008</td>
<td>6</td>
<td>415 ± 53</td>
<td>232–617</td>
</tr>
<tr>
<td>Tashtagol</td>
<td>13.05.2008</td>
<td>5</td>
<td>200 ± 42</td>
<td>101–334</td>
</tr>
<tr>
<td>Tashtagol</td>
<td>04.02.2009</td>
<td>7</td>
<td>730 ± 77</td>
<td>192–1285</td>
</tr>
<tr>
<td>Tashtagol</td>
<td>11.02.2009</td>
<td>22</td>
<td>441 ± 88</td>
<td>110–1373</td>
</tr>
<tr>
<td>Tashtagol</td>
<td>02.03.2010</td>
<td>10</td>
<td>905 ± 134</td>
<td>680–1143</td>
</tr>
<tr>
<td>Tashtagol</td>
<td>02.03.2011</td>
<td>18</td>
<td>347 ± 101</td>
<td>74–749</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Krasnoye village</td>
<td>25.01.2008</td>
<td>12</td>
<td>106 ± 18</td>
<td>39–203</td>
</tr>
<tr>
<td>Pacha village</td>
<td>16.05.2008</td>
<td>6</td>
<td>64 ± 22</td>
<td>20–135</td>
</tr>
<tr>
<td>Zarubino village</td>
<td>14.03.2011</td>
<td>10</td>
<td>119 ± 33</td>
<td>39–203</td>
</tr>
<tr>
<td>Zarubino village</td>
<td>23.01.2011</td>
<td>10</td>
<td>64 ± 13</td>
<td>39–203</td>
</tr>
<tr>
<td>Zarubino village</td>
<td>06.04.2011</td>
<td>17</td>
<td>119 ± 27</td>
<td>53–172</td>
</tr>
</tbody>
</table>
the supernatant was removed, the pellet was resuspended. The pellets were placed in a hypotonic solution of 0.55% KCl for 10–15 min at 37°C. The fixation of the material was performed in cooled fresh Carnoy’s fixative (methanol and acetic acid in the ratio 3:1). The cell suspension was pipetted onto clean, cooled slides moistened with water. The preparations were encoded and stained with 2% Giemsa solution.

Counting of the aberrations was performed using light microscopy at ×1000 magnification (oil immersion) without karyotyping. The selection of metaphases included in analysis and criteria for cytogenetic abnormalities conformed to the generally accepted recommendations (20). We identified these cytogenetic abnormalities as, frequency of aberrant cells (%), frequency of aberrations/100 cells (%), average frequencies of single and double fragments (%), frequency of chromatid and chromosome interchanges (%), frequency of dicentric chromosomes with fragments (%), frequency of ring chromosomes (%) and translocations (%), number of aberrations chromatid and chromosome type (%), and rogue cells (%).

Assessment of the background mutagenic activity was performed using standard methods: the Ames test (21) and the assay for dominant lethal mutations (22).

### Table 2. Gender and age of children/teenagers included in the exposed and control groups

<table>
<thead>
<tr>
<th>Person</th>
<th>Number</th>
<th>Age (mean ± SD)</th>
<th>Age (min–max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exposed group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>372</td>
<td>12.24 ± 2.60</td>
<td>8–18</td>
</tr>
<tr>
<td>Male</td>
<td>195</td>
<td>12.14 ± 2.63</td>
<td>8–18</td>
</tr>
<tr>
<td>Female</td>
<td>177</td>
<td>12.36 ± 2.58</td>
<td>8–18</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>186</td>
<td>14.43 ± 2.58</td>
<td>8–19</td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>13.90 ± 2.58</td>
<td>9–19</td>
</tr>
<tr>
<td>Female</td>
<td>103</td>
<td>14.85 ± 2.51</td>
<td>8–19</td>
</tr>
</tbody>
</table>

### Table 3. Results of the assessment of mutagenic activity in the air probes (the Ames test)

<table>
<thead>
<tr>
<th>Conditional sample volume, m³/Petri dish</th>
<th>The rate of excess average number of Salmonella typhimurium colonies per Petri dish in the experience to the negative control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krasnoye village</td>
<td>TA98</td>
</tr>
<tr>
<td>0.04</td>
<td>1.23</td>
</tr>
<tr>
<td>0.11</td>
<td>1.21</td>
</tr>
<tr>
<td>0.32</td>
<td>0.98</td>
</tr>
<tr>
<td>0.96</td>
<td>1.17</td>
</tr>
<tr>
<td>Tashtagol</td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>1.29</td>
</tr>
<tr>
<td>0.11</td>
<td>1.46</td>
</tr>
<tr>
<td>0.32</td>
<td>1.25</td>
</tr>
<tr>
<td>0.96</td>
<td>0.90</td>
</tr>
<tr>
<td>Positive control</td>
<td>Ethidium bromide</td>
</tr>
<tr>
<td></td>
<td>DDDTP (C₅H₅N₄O₄)</td>
</tr>
<tr>
<td></td>
<td>Sodium azide</td>
</tr>
<tr>
<td>Negative control (the average number of colonies per dish)</td>
<td>5% DMSO</td>
</tr>
</tbody>
</table>

DMSO, dimethyl sulphoxide.

*Significant mutagenic effect.

### Results

The average volume radon activity in the residential areas of the children of exposed group was $468 ± 77$ Bq/m³ during all years of the investigation. It exceeds the similar parameter for the control group ($94 ± 23$ Bq/m³). Gamma background (from natural sources of radiation) in the exposed group was 11–18 microroentgen/hour (control group: 12–14 microroentgen/hour). The individual effective dose inhalation exposure due to isotopes of radon and its short-lived decay products was ~27 mSv/year. Full results of the measurements of volume radon activity are presented in Table 1.

Air probes for the Ames test showed not mutagenic effects on cultures of *Salmonella typhimurium* TA98 and TA100. Full results are presented in Table 3. Test for the induction of dominant lethal mutations in *Drosophila melanogaster* showed an increased level of early embryonic lethality in the air probes from Tashtagol, which explains to occurrence of some agents that influence the physiological features of fly embryonic growth. Full results are presented in Table 4. The number of eggs laid after exposure to water from Tashtagol and Krasnoye village did not differ (23).

Results of the chromosome aberration studies in the cohort of long-term resident children under conditions of high radon exposure and the control group are presented in Table 5.

The primary cytogenetic indicator, the frequency of aberrant cells, was significantly increased in the exposed group (4.26 ± 2.47 vs. 2.74 ± 1.64%—in control group). In addition, there were also 5% DMSO.

### Statistical analysis

Statistical analysis was performed using the program StatSoft STATISTICA 6.0. We used the Kolmogorov–Smirnov test to verify the compliance of the data with the normal distribution. The data analysis was performed using the non-parametric statistics block. Group comparisons were performed using the *U*-rank Mann–Whitney test. Spearman’s correlation coefficient was used to calculate the correlation.
significant increases of the frequencies of single and double fragments (3.02 ± 2.20 vs. 2.05 ± 1.26% and 1.12 ± 0.94 vs. 0.66 ± 0.78%, respectively), chromosome interchanges (0.22 ± 0.43 vs. 0.06 ± 0.18%), number of aberrations chromatid and chromosome type (3.04 ± 2.20 vs. 2.08 ± 1.28% and 1.33 ± 1.09 vs. 0.72 ± 0.79%) and a nearly 2-fold increase of the frequency of aberrations/100 cells (4.38 ± 2.57 vs. 2.80 ± 1.71%) in the exposed groups. The percentage of chromatid interchanges in the control group exceeded this value (4.38 ± 2.57 vs. 2.47 ± 2.69% and 2.47 ± 2.69 vs. 2.57 ± 2.80). The percentage of chromosomes with fragment (0.79 ± 0.78%) and 0.78% (0.79 ± 0.78% vs. 0.78%) in the exposed group, but this was not significant (P = 0.651). The remaining indicators showed an increase in the exposed group, but these differences were also not significant (Table 5).

Sex does not influence the frequency of any type of chromosome abnormalities.

We identified a significant increase of the frequencies of double fragments (1.28 ± 1.06 vs. 0.99 ± 0.82%, P = 0.020) and aberrations chromosome type (1.49 ± 1.19 vs. 1.21 ± 0.99%, P = 0.049) in the second childhood group (9–12 years old boys, 9–11 years old girls) compared to the first childhood (4–8 years old) in the control and exposed groups. There is trend towards the increasing frequency of double fragments in the older age group from Tashtagol. There was a tendency to positive correlation for the duration of residence under high radon conditions with the total frequency of aberrations (Figure 1A), with frequency of double fragments (Figure 1B) and with the aberrant chromosome type (Figure 1C). We found significant differences between smoking (58 people) and non-smoking (314 people) children for several cytogenetic parameters in the exposed group. We eliminated smoking children from the study cohort to exclude the influence of smoking and performed the comparison of non-smoking children. We showed that smoking does not significantly influence the differences between groups but lead to an increase in the level of total aberrations (Table 3). This is evidence of the leading role of radiation by radon and its decay products in the increased level of cytogenetic abnormalities in the exposed group.

### Discussion

Based on the presented data, the radon level in the indoor air of a boarding school during the winter period exceeded the permissible level (up to 200 Bq/m³) for operating buildings. We also found a marked decrease of radon volume activity during spring, but even under conditions of improved ventilation, the radon concentration remained relatively high (Table 2). The average radon volume activity in the living rooms of the exposed group ranged from 235 to 905 Bq/m³ in the winter and from 200 to 347 Bq/m³ in the spring. The average value of the radon volume activity over 5 years was 463 ± 98 Bq/m³, which is much higher than the control settlements.

Therefore, the living and education conditions in the boarding school of Tashtagol do not correspond to the radiation safety standards, and this group is chronically exposed to excessive doses of radon, which can induce genotoxic effects.
A number of studies showed the genotoxic risk, including clastogenic, of radon exposure. Uranium miners or other miners exposed to high doses of radon emission are characterised by a higher level of cytogenetic damage in their peripheral blood lymphocytes compared to control groups (24–26). In addition, the high values of aberrant metaphases and the high frequency of chromatid breaks in miners are significantly associated with cancer risk (27).

On the other hand, some articles (28–33) show the opposite relationship between radon exposure and the level of DNA damage and the cancer risk. This fact is evidence of the lack of knowledge about this problem and the necessity for more extensive research.

In contrast to occupational exposure, the effects of radon exposure on the frequency of chromosome aberrations in residential conditions are contradictory. Possible reasons for this are a relatively low concentration of radon in dwellings and a limited number of individuals surveyed (34–36). A study conducted in the UK showed a significant association between the frequency of mutations at the hprt-locus and indoor air radon concentration. However, in subsequent studies using the expanded sample and determining the cytogenetic translocations for BCL-2, there was no correlation between the frequency of mutations and radon level (37). Swedish researchers used a DNA-comet assay to assess the primary DNA damage in single cells. They analysed the blood samples from 125 people living in 45 houses with different indoor air radon concentrations (35–1025 Bq/m³), as well as with different concentrations of radon in the drinking water (10–2410 Bq/l). The increased radon concentration in the air (>200 Bq/m³) was associated with increased DNA damage in lymphocytes (P < 0.05). There was also no correlation between radon concentration in the drinking water and the level of cytogenetic damage (38). Other studies used fluorescence in situ hybridization technology to assess the induction of stable chromosome interchanges in a sample of residents in homes with different radon concentrations (27,38,39). Virtually none of the cited publications found significant increases of translocation frequency in the persons living in conditions of very high air radon concentrations. However, the frequency of unstable chromosome interchanges, such as dicentric and ring chromosomes, was significantly increased in people exposed to high radon concentrations (200 Bq/m³) compared to a control group (2.45 ± 0.50 and 1.03 ± 0.15%; P < 0.05) (34).

The effectiveness of cytogenetic tests to assess clastogenic effects induced by increased radon concentrations in indoor air has been shown in several studies. Interestingly, the proportion of aberrant metaphases in the sample studied here was nearly twice as many as in the study from Slovenia (40) (4.26 vs. 2.03%).

Figure 1. Correlations of the total frequency of aberrations (A), double fragments (B) and aberrations chromosome type (C) with duration of residents in conditions of high radon concentration and its decay products.

Notably, the duration of the stay of the surveyed children in the boarding school can reach 18–20 h a day, particularly during the cold period that lasts for 5–6 months in the continental climate of Western Siberia. The wintertime radon concentration is the highest because of the poor ventilation and energy-saving technologies used. The increased frequency of dicentric and ring chromosomes under conditions of radon exposure from 0.043 to 0.35% (41) and from 0.15 to 0.44% (42), respectively. In our study, we found that the frequency of dicentric and ring chromosomes increasing in the exposed group, but was not statistically significant. The overall
frequency of chromosome interchanges, including dicentric chromosomes, ring chromosomes and translocations, differentiable by routine staining in the exposed group was significantly higher than in controls (0.21 ± 0.42 and 0.05 ± 0.18%, respectively, P < 0.05).

Chromosome interchanges are often used as markers of ionising radiation exposure. In this study, we found no significant effect of other factors capable of modifying the chromosome aberration frequency, such as the action of chemical mutagens, morbidity, sex and age. Assessing the combined influence of radon exposure and smoking showed that the average frequency of atomic fragments and chromatid type aberrations under conditions of the combined influence of these factors were significantly increased compared with the group exposed to radon only (Table 5). Our findings are consistent with the available scientific publications. Recent studies showed that the percentage of acentric fragments and chromatid type aberrations under conditions of combined radon (189 Bq/m^3) and smoking exposure were significantly (P < 0.0001) increased compared to the control (non-smoking individuals) (1.63 ± 0.87 and 1.41 ± 1.04% vs. 0.926 ± 0.67 and 0.827 ± 0.66%, respectively) (43).

Conclusion

We found that long-term resident children under conditions of high exposure to radon and its decay products are characterised by an increased frequency of several cytogenetic abnormalities, including chromosome interchanges that occurred almost four times often as the control group. It should be emphasised that among these abnormalities, we found dicentric and ring chromosomes, often regarded as specific markers of radiation exposure. We also found a modifying effect of smoking and residence duration under conditions of low-level ionising radiation exposure on the incidence of these damages. Therefore, the method of chromosome aberration analysis showed high efficiency for the evaluation of chronic low-level ionising radiation exposure on children and adolescents under domestic conditions.

Funding

State task (No. 2015/2162).

Acknowledgements

We are particularly indebted to leading researcher Dr Finia I. Ingel (Institute of Human Ecology and Environmental Hygiene A.N. Sysina, Moscow, Russian Federation) for their help in performing of the fruit fly mutation measurements; to Dr Lyudmila V. Ahaltsseva (Institute of Human Ecology and Environmental Hygiene A.N. Sysina, Moscow, Russian Federation) for their help in performing of the Aims test; to Dr Natalia V. Sorokina (Kemerovo State University, Kemerovo, Russian Federation) for his help in the performing of radiological investigations.

Conflict of interest statement: None declared.

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