ANALYSIS OF UNSTABLE CHROMOSOME ALTERATIONS FREQUENCY INDUCED BY NEUTRON-GAMMA MIXED FIELD RADIATION

Priscilla L. G. Souza¹, José Odinilson de C. Brandão¹², Merilane S. Calixto³, Carlos H.F.P. Vale¹, Joelan A. L. Santos¹², Neide Santos³, Eudice C. Vilela¹ and Fabiana F. Lima¹

¹Centro Regional de Ciências Nucleares (CRCN-NE/CNEN-PE)
Av. Professor Luiz Freire, 200
50740-540 Recife, PE
psouza@cnen.gov.br
jodinilson@cnen.gov.br

²Departamento de Energia Nuclear (DEN/UFPE)
Av. Professor Luiz Freire, 1000
50740 Recife, PE

³Departamento de Genética (DG/UFPE)
Av. Prof. Moraes Rego, 1235
50670-901, Recife - PE

ABSTRACT

Nowadays monitoring chromosome alterations in peripheral blood lymphocytes have been used to access the radiation absorbed dose in individuals exposed accidental or occupationally to gamma radiation. However there are not many studies based on the effects of mixed field neutron-gamma. The radiobiology of neutrons has great importance because in nuclear factories worldwide there are several hundred thousand individuals monitored as potentially receiving doses of neutron. In this paper it was observed the frequencies of unstable chromosome alterations induced by a gamma-neutron mixed field. Blood was obtained from one healthy donor and exposed to mixed field neutron-gamma sources $^{241}$AmBe (20 Ci) at the Neutron Calibration Laboratory (NCL – CRCN/NE – PE – Brazil). The chromosomes were observed at metaphase, following colcemid accumulation and 1000 well-spread metaphases were analyzed for the presence of chromosome alterations by two experienced scorers. The results suggest that there is the possibility of a directly proportional relationship between absorbed dose of neutron-gamma mixed field radiation and the frequency of unstable chromosome alterations analyzed in this paper.

1. INTRODUCTION

A significant number of people have the potential to be occupationally exposed over a protracted period to low doses of neutrons as occupational doses due to photoneutrons at the vicinity of linear accelerators. In addition, there is increasing concern about airline crew members (about one million worldwide) are exposed to measurable neutrons doses [1]. As consequence of such exposure, raises the possibility of cancer and leukemia plus hereditary mutations [2].
Neutron irradiation of living cells leads to the induction of the same biological end-points as gamma or X radiation, however, have been previously explained that neutrons cause less physiological damage, such as changes in the surface properties of chromosomes and more genetic damage, such as chromosome alterations [3].

Historically, cytogenetic biodosimetry assays have been used to identify the absorbed dose. This method is based on quantifying of asymmetrical chromosome alterations (dicentrics, centric rings and acentric fragments) in mytogen-stimulated T-lymphocytes in their first mitosis after radiation exposure [4].

Increased levels of chromosome damage in peripheral blood lymphocytes are a sensitive indicator of radiation exposure and they are routinely exploited for assessing radiation absorbed dose after accidental or occupational exposure. In addition, it may be possible to predict the future effect on health by a long-term follow-up study of radiation effects [6]. It has been shown in prospective follow-up studies [5,7,3] that subjects with elevated levels of structural chromosomal aberrations (CAs) may be at an elevated risk for cancer, whereas no such association was observed with other biomarkers. This places the CAs assay as a reliable indicator of risk. On the other hand, cytogenetic biomarkers as a whole can be helpful in evaluating the in vitro and in vivo mechanisms underlying DNA damage from ionizing radiation [3].

In this paper, it was observed the frequencies of unstable chromosome alterations (dicentric, acentric fragments and centric rings) irradiated by a mixed field neutron-gamma source ($^{241}$AmBe) at 2 different doses.

2. MATERIALS AND METHODS

2.1 Blood samples

Heparinised blood (10 mL) was taken from one healthy individual, male, and 24 years old, at the Laboratory of Biological Dosimetry (LBD – CRCN/NE CNEN – PE – Brazil). For each dose, the sample was divided equally (5mL + 5mL) between two culture tubes. One of them was irradiated and another kept at room temperature (~20 °C).

2.2 Irradiation

Blood was exposed to two mixed field neutron-gamma sources $^{241}$AmBe (20 Ci) at the Neutron Calibration Laboratory (NCL – CRCN/NE – PE – Brazil). The distance between the sources and the sample was 3.75 cm (the centre of the sample was assumed to be the geometrical centre of the liquid in the tube, taking as main axis the one perpendicular to the beam direction). The polyethylene barrier (2mm) was used to ensure the electronic equilibrium. At the irradiation position, spectrum was determinate using a Bonner Sphere System manufactured by LUDLUM Measurements Inc., model 42-5. The samples received doses of 0.41 and 0.96 Gy. Immediately after exposure, both of blood samples were cultured.
2.3 Cell culture

From each sample, two blood cultures were set up. Lymphocytes were cultured for 48 hours in RPMI 1640 medium (Sigma), supplemented with 20% (v/v) fetal calf serum, 1% (v/v) phytohemaglutinin (Biological Industries), 1% Hepes (v/v) and 50 µg/mL streptomycin. 0.05 µg/mL Colcemid (Biological Industries) was added 46 hours after culture started and incubation continued for two more hours. The cells were harvested by centrifugation of the samples and the cell pellets were resuspended in 0.075 M KCl and kept for 15 min at 37°C. After the hypotonic shock the cells were fixed 3 times in methanol:acetic acid (3:1). Finally, cells were dropped on clean slides and stained with a 5% Giemsa solution (Merk). At least 1000 well-spread metaphases were analyzed per groups (controls and irradiated) for the presence of unstable chromosome alterations by two experienced scorers.

2.4 Scoring criteria

Scoring of chromosomal aberrations (Fig. 1) was performed directly at the optical microscope (Edutec 502 AC). Only cells at the first cycle complete metaphases (colcemid blocked) were scored. The slide was examined in its entirety and only viable metaphases were counted. It is understood by viable metaphases those which showed no overlapping chromosomes, with 46 centromeres.

Fig. 1. Metaphase observed under optical microscopy containing one dicentric chromosome (arrow in the middle) and two acentric fragments (arrows remaining) – total magnification: 1600x.
3. RESULTS AND DISCUSSION

The chromosome aberrations observed in human peripheral blood sample, exposed for 6 and 14 hours to a neutron-gamma mixed field were counted, according to the previously reviewed experimental arrangement. More than 4,000 metaphases were analyzed. The number of unstable chromosome alterations scored and their frequencies are presented in Table 1 for the irradiated and control groups.

Table 1. Unstable chromosome alterations scoring results observed under optical microscopy (total magnification: 1600X) for the different irradiation doses.

<table>
<thead>
<tr>
<th>Chromosome Alterations</th>
<th>Irradiated blood</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.41Gy</td>
<td>0.96Gy</td>
</tr>
<tr>
<td>AN^a</td>
<td>33</td>
<td>95</td>
</tr>
<tr>
<td>F^b</td>
<td>0.0321</td>
<td>0.0929</td>
</tr>
<tr>
<td>Associated Dicentrics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Associated Fragments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dicentric</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Fragments</td>
<td>20</td>
<td>95</td>
</tr>
<tr>
<td>Centric rings</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>TOTAL METAPHASES SCORED</td>
<td>1027</td>
<td>1022</td>
</tr>
</tbody>
</table>

a. Total number of alterations; b. Frequency of cellular alterations.

Table 1 showed the distribution and frequency of chromosome alterations in control samples and in samples exposed at a dose of 0.41Gy and at 0.96Gy. The frequency of associated dicentrics increased from 0.0321 to 0.0929 per cell by increasing the dose and frequency of centric rings increased similarly – from 0.0097 to 0.0303. In addition, the frequency of fragments showed a further increase from 0.0194 to 0.0929.

According to Lloyd et al. (1976) [8], in samples irradiated with 0.54 Gy and 1.08 Gy were observed significantly different frequencies for unstable chromosome aberrations as shown in table 2.
Table 2. Frequencies of unstable chromosome alterations founded by Lloyd et al. (1976) for neutrons with energy of 7.6MeV.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>Cells Scored</th>
<th>Frequency Dicentrics</th>
<th>Frequency Acentric Rings</th>
<th>Frequency Acentrics</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.54</td>
<td>342</td>
<td>0.2807</td>
<td>0.0263</td>
<td>0.2807</td>
</tr>
<tr>
<td>1.08</td>
<td>143</td>
<td>0.6993</td>
<td>0.0279</td>
<td>0.4405</td>
</tr>
</tbody>
</table>

However, large difference in these frequencies can be attributed to the fact the mentioned paper have used a mixed field neutron-gamma with mean energy of 7.6 MeV which gamma vector corresponds to approximately 10% of the radiation beam, while in this paper the mean energy used was 4.4 MeV which gamma vector corresponded to 50% of the radiation beam, moreover these doses are different from those we performed. Another important fact to be considered is the number of cells counted per dose is too below the recommended number of cells analyzed by the International Atomic Energy Agency [9] and used in our paper, which corresponds to a minimum of 1000 cells.

The determination of the frequency of dicentric chromosomes has been used for biological dosimetry since the early 1960s. Dicentric aberrations or more complex polycentric chromosomes develop when chromosome fragments are exchanged in such a way that two fragments, each containing a centromere, are fused. Since 50% of these types of aberrations are eliminated at each mitosis, the cells have to be examined at their first division 46–48 h after initiation of the culture.

The principal advantage of determining dicentric and ring chromosomes is the relatively easy and good standardization of technique and evaluation in doing so and, therefore, this forms the method of choice for most suspected cases of accidental overexposure when the analysis can be performed within months after exposure. Generally, 100–500 cells should be scored to evaluate a medium or high exposure with a satisfactory accuracy. In order to reliably detect an exposure of 10 mSv, however, a minimum of 10,000 cells will have to be analyzed. An exposure to radiation of several Sv drastically reduces the number of lymphocytes in the blood, but since the number of aberrations per cell is high in such cases, scoring of a small number of cells, even of a few tens, may suffice for an acceptable dose estimate. The technique is expensive, requires well-trained personnel and is unsuitable when a period of years has elapsed after exposure because of the limited lifespan of unstable aberrations [10].

So, according to Littlefield (2000) [4], it is important to study continuously the cytogenetic effects induced by the type of radiation in question, since there are few data available in literature and it’s increasing the number of exposed individuals.
4. CONCLUSIONS

The results obtained show that there may be a relationship between the absorbed dose and frequency of all unstable chromosomal alterations analyzed and each alteration presents a characteristic frequency.

The results also suggest that there is the possibility of using different types of chromosome alterations to achieve the biological dosimetry of neutron-gamma mixed field.

ACKNOWLEDGMENTS

Authors would like to acknowledge CNPq for the financial support.

REFERENCES