

DEVIATIONS FROM POISSON STATISTICS OBSERVED IN CHROMOSOME ABERRATIONS INDUCED BY ^{252}Cf NEUTRONS*

W. PEREIRA^a, A. KOWALSKA^b, K. CZERSKI^a, E. NASONOVA^c
P. KUSTALO^c, L.E. VALERIEVICH^d

^aUniversity of Szczecin, Institute of Physics, 70-451 Szczecin, Poland

^bMaritime University of Szczecin, Inst. of Mathematics, Physics and Chemistry
70-500 Szczecin, Poland

^cJoint Institute for Nuclear Research, Laboratory of Radiation Biology
Dubna, Russia

^dJoint Institute for Nuclear Research, Frank Laboratory of Neutron Physics
Dubna, Russia

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Biological efficiency of ion beams and neutral particles can be determined by studying the number of chromosome aberrations induced in irradiated cells. In the present investigation, we studied Relative Biological Efficiency (RBE) and statistical distributions of chromosome aberrations induced in human peripheral blood lymphocytes exposed to 0.23 Gy and 0.47 Gy of ^{252}Cf neutrons. Due to the possible cell cycle delay, the aberrations were scored at two different sampling times. Data were fitted using Poisson and Generalized Poisson distributions. In all cases, experimentally measured distributions significantly deviate from theoretical expectations.

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1. Introduction

Possible applications of neutrons in radiotherapy have been studied since shortly after the discovery of the neutron by Chadwick in 1932, starting with an early clinical work of Ref. [1]. Presently, we use two types of cancer radiotherapy with neutrons: fast neutron therapy and Boron Neutron Capture Therapy (BNCT) using epithermal neutrons. In both cases, understanding of a cell response function to neutron irradiation is still a limiting factor for

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further development of the cancer treatment. The study of biological efficiency of ion beams and neutral particles is of great interest for medicine due to the extending use of hadron therapy for cancer treatment. In our previous works, we investigated induction of chromosomal damage in peripheral human blood lymphocytes exposed to gamma radiation as well as to protons and heavy ions [2, 3], showing that the repair mechanisms are mainly responsible for the non-linear response function observed experimentally [4].

Since neutrons are uncharged particles, they do not ionize the target material and lead to biological damage, directly. Depending on neutron energy, different processes can play a dominant role: elastic and non-elastic scattering of neutrons, and neutron-induced reactions. The latter is used in BNCT which is a two-step procedure. First, the patient is injected with a tumor-localizing drug containing non-radioactive ^{10}B that has a high propensity to capture slow neutrons. In the second step, the patient is exposed to epithermal neutrons, which are absorbed by ^{10}B and induce emission of high-energy α particles, thereby killing the cancer cells in the immediate vicinity [5].

In the present work, chromosome aberrations in human lymphocytes exposed to neutron irradiation are studied by use of neutrons emitted in spontaneous fission of a ^{252}Cf source. Special attention is paid to experimentally determined distributions of the aberration frequency, for which indications of an increased induction of multiple aberrations have been found in previous works [6–8]. Similar effects were also observed for human cells irradiated by heavy ions [2, 9], which could be explained by the ion track structure induced by swift heavy ions. Thus, to describe that effect, we will utilize Generalized Poisson Distribution [10] which allows for fitting of an additional parameter compared to the standard Poisson Distribution, which is expected to account for overproduction of multiple chromosome aberrations.

2. Materials and methods

The experiments were carried out at the Frank Laboratory of Neutron Physics, JINR, Dubna, Russia. A ^{252}Cf source was used, providing neutrons in a 4π geometry with a continuum spectrum (see Fig. 1) and average energy of 2.12 MeV. The whole blood samples were obtained according to the ethical regulations of the Russian Federation by venipuncture from a young healthy male volunteer. The ^{252}Cf source, placed in a stainless-steel container of 3 mm diameter, was inserted into the round-bottom Eppendorf's tubes of 10 mm diameter which contained 0.5 ml of blood. Exposure was performed at room temperature, samples were sham-irradiated. After the exposure, the blood was diluted in 4.5 ml of the nutrient medium (RPMI supplemented by 20% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin and 1.5% phytohaemagglutinin (PHA)); reagents

were from Sigma-Adrich and GIBCO). All procedures were done according to the recommendations of IAEA [11]. The samples were incubated at 37°C and 5% CO₂, and fixed at 48 h and 72 h after PHA stimulation since it was previously demonstrated by one of the authors [2] that high-LET radiation (in contrast to low-LET ones) effectively induces cell cycle delay of heavily damaged cells, and they are able to reach mitosis at later postirradiation time as compared to those exposed to photons. For the dose calculation and spectrum evaluation, a Monte Carlo simulation using the computer code Geant4 [12] was performed.

In Fig. 1, the simulated energy spectrum of neutrons emitted directly from the radioactive source is compared to the simulated spectrum of neutrons that traversed a blood sample.

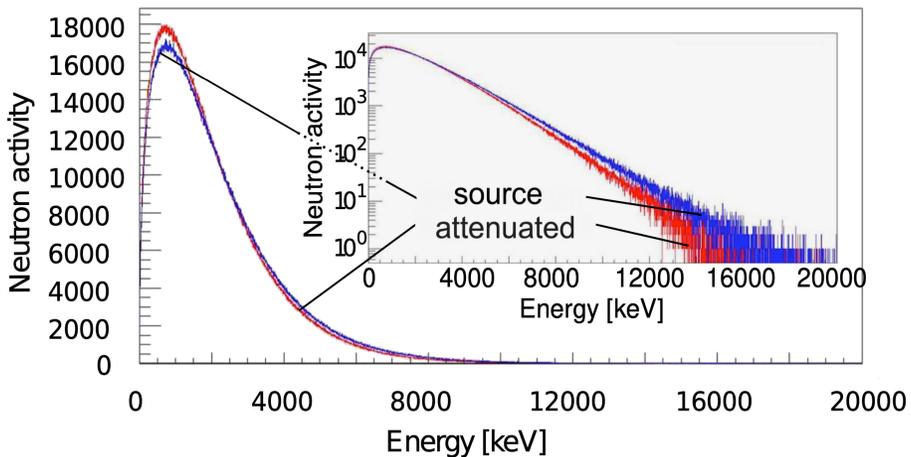


Fig. 1. (Color online) Spectra of ²⁵²Cf neutrons simulated using Geant4. In dark grey/blue, the actual source spectrum, and in light grey/red, a spectrum of neutrons that passed through a blood sample.

Experimentally determined frequency distributions of chromosome aberrations can be studied by means of the Poisson Distribution (PD)

$$P_p(k) = \frac{\lambda_p^k e^{-\lambda_p}}{k!},$$

where λ_p represents the mean number of aberrations observed per cell after exposition to a given dose of ionizing radiation, and k corresponds to the current number of aberrations per cell. Since the experimental data clearly deviate from the pure PD, the Generalized Poisson Distribution (GPD) was used. GPD assumes that the deviations from PD are due to competing processes that can influence both induction and repair of DNA damages and

can be described by a single parameter θ [10]. The probability distribution of GPD reads as follows:

$$P_{\text{GPD}}(k, \theta) = \frac{\lambda_p(\lambda_p + \theta k)^{k-1} e^{-\lambda_p - \theta k}}{k!}, \quad k = 0, 1, 2, 3, \dots$$

When the θ parameter is equal to 0, the process follows the Poisson law. A positive θ value is explained as a result of additional mechanisms leading to an increased number of aberrations — the observed frequency distribution is then said to be over-dispersed. When θ is negative, the number of aberrations is reduced due to repair mechanisms and the observed distribution is said to be under-dispersed.

3. Results and statistics

Distributions of chromosome aberrations induced in human peripheral blood lymphocytes after exposure to 0.23 Gy and 0.47 Gy of ^{252}Cf neutrons are presented in Fig. 2. For each dose, 100 cells were scored.

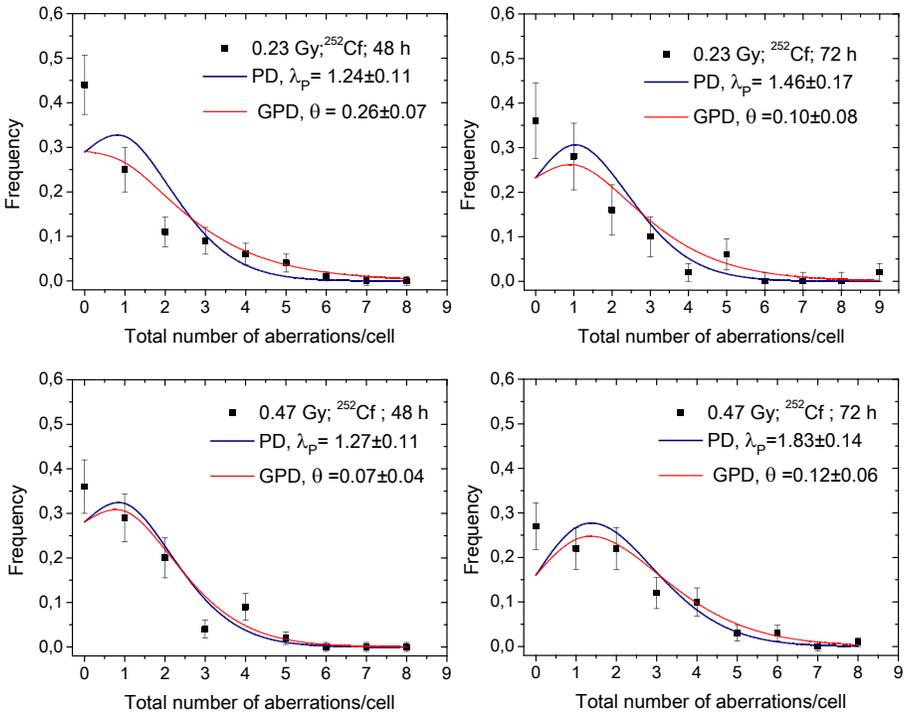


Fig. 2. (Color online) Poisson (grey/red line) and Generalized Poisson Distribution (black/blue line) of chromosome aberrations induced by 0.23 Gy and 0.47 Gy neutron irradiation, observed after 48 h and 72 h of incubation.

In both cases, due to possible occurrence of cell cycle delay [13], aberrations were observed in two postirradiation times (48 h and 72 h). In the case of 0.23 Gy, mean number of aberrations per cell, represented by the λ_p parameter was 1.24 ± 0.11 after 48 h and increased to 1.46 ± 0.14 (single standard deviation) after 72 h of incubation. In comparison, θ parameter of GPD decreased in time from 0.26 ± 0.07 to 0.10 ± 0.08 .

In the case of 0.47 Gy irradiation, λ_p increased with incubation time more significantly, reaching 1.27 ± 0.11 after 48 h and 1.83 ± 0.17 for 72 h. The parameter θ took on relatively low values of 0.074 ± 0.04 and 0.12 ± 0.06 for the incubation time of 48 h and 72 h, respectively. In all studied cases, both Poisson and Generalized Poisson distributions underestimate the frequency of cells without aberrations. The Relative Biological Effectiveness (RBE) values were calculated as a ratio of isoeffective doses of neutrons and ^{60}Co γ rays (data obtained for gamma radiation were taken from Ref. [3]). For 72 h incubation time, RBE is equal to ~ 5.6 for the higher dose and ~ 10.0 for the lower one. Due to the cell cycle delay effect evidenced by Fig. 2, only the values obtained for 72 h incubation time are meaningful.

4. Discussion and conclusion

Chromosome aberrations are considered to be a very sensitive bioindicator of radiation action. High biological efficiency of neutrons is very important for neutron cancer therapy, which is mainly advantageous for the treatment of large, deep-seated tumors [15]. Due to high RBE value, the treatment process can be significantly reduced. Biological interaction of neutrons is also very interesting with regard to safety of the hadron radiotherapy, since highly energetic neutrons are produced in the absorbers, range shifters, tissue equivalent materials or even in the patient's body through nuclear reactions of charged particles [7, 14]. Finally, studies of biological efficiency of neutrons and other radiation species in combination with the repair mechanisms are essential for radiation protection due to growing interest of nuclear power. In the present study, we have collected data on chromosome aberrations induced by ^{252}Cf neutrons with a continuum energy spectrum and average energy 2.12 MeV at doses of 0.23 Gy and 0.47 Gy estimated using a **Geant4** simulation. We have studied both RBE and statistical frequency distributions of chromosome aberrations. The RBE values show a clear dose and incubation time dependence. As expected, RBE amounts higher values for the lower dose and the longer incubation time when heavily damaged cells reach mitosis. It was already reported [16] that exposure of cells to high-LET radiation delays the cell cycle progression and may lead to underestimation of the damage observed in lymphocytes collected at the standard fixation time of 48 h. The mean number of aberrations per cell (λ_p)

is almost equal for both studied doses, when measured after 48 h incubation (the exact values are 1.24 ± 0.11 and 1.27 ± 0.11 at 0.23 Gy and 0.47 Gy, respectively). After 72 h of incubation, these values increase to 1.46 ± 0.14 and 1.83 ± 0.17 , respectively. Thus, the higher is the dose, the larger is the number of heavily damaged cells with delayed entry into mitosis. The Poisson distribution cannot, however, correctly describe the experimental data. The Generalized Poisson Distribution, fitted with one free parameter that should account for additional processes changing the aberration number, provides slightly better results. Nevertheless, in both cases, we observe a significant underestimation of the number of chromosomes without any aberrations. Moreover, the positive value of the parameter θ would indicate that the Poisson distribution systematically underestimates also the number of multiple aberrations. It would mean that there is an additional mechanism responsible for the larger aberration numbers. This effect can be probably explained by nuclear reactions (*e.g.* (n, α)) that may take place in irradiated samples [17]. However, further studies are certainly necessary.

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