STATISTICAL EFFECTS OF THE REPAIR MECHANISMS OF CELLS DAMAGED BY IONIZING RADIATION

STATYSTYCZNE EFEKTY REPERACJI KOMÓREK USZKODZONYCH W WYNIKU NAŚWIETLENIA PROMIENIOWANIEM JONIZUJĄCYM

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ABSTRACT

Ionizing radiation passing through living matter in the form of ion tracks causes DNA double strand breaks, which lead to chromosomal aberrations. On one hand, the number of aberrations is determined by the absorbed dose of radiation and on the other hand results from the efficiency of the repair mechanisms. These mechanisms are especially important for low doses, the study of which is particularly time-consuming.Here, a new method to estimate the effectiveness of repair mechanisms is presented. The method is based on the reduction of variance of observed chromosomal aberrations in relation to the values expected for the Poisson statistics. This effect is known in other fields of physics as the Fano factor and can be applied to various tissues and for both low and high LET (Linear Energy Transfer) radiation's types.By exploiting the new method, distributions of chromosomal aberrations in peripheral blood lymphocytes observed for gamma and 150 MeV proton radiation, have been compared. Experiments using proton beams were carried out in the Joint Institute for Nuclear Research in Dubna, Russia.

Keywords:chromosomal aberrations, repair mechanisms, Poisson statistics, Fano factor

STRESZCZENIE

Promieniowanie jonizujące, tworząc w materii ożywionej ślady jonowe i łamiąc podwójną helisę DNA, prowadzi do powstania aberracji chromosomowych. Ich liczba z jednej strony określona jest pochłoniętą dawką rodzajem promieniowania, a z drugiej strony wynika z wydajności mechanizmów reperacji komórkowej. Mechanizmy te są szczególnie ważne w przypadku niskich dawek, dla których badania są szczególnie pracochłonne.W niniejszej pracy przedstawiona zostanie metoda oszacowania efektywności mechanizmów reparacyjnych w oparciu o obserwowaną redukcję wariancji liczby otrzymanych aberracji chromosomowych w stosunku do zakładanej wartości według statystyki Poissona. Efekt ten znany jest w innych dziedzinach fizyki

jako czynnik Fano i może być zastosowany do różnych tkanek oraz promieniowania jonizującego o niskim bądź wysokim LET (ang. *LinearEnergy Transfer*).W oparciu o nową metodę statystycznego oszacowania efektywności mechanizmów reperacji komórkowej porównane zostaną rozkłady aberracji chromosomów limfocytów krwi obwodowej obserwowane dla naświetlań zarówno kwantami gamma, jak i protonami o energii 150 MeV. Eksperymenty z użyciem wiązki protonowej przeprowadzone zostały w Zjednoczonym Instytucie Badań Jądrowych w Dubnej w Rosji.

Słowa kluczowe: aberracje chromosomowe, mechanizmy reparacyjne, statystyka Poissona, czynnik Fano

1. Introduction

The wide use of heavy ion cancer therapy boosts the experimental studies on DNA damage and repair processes. The investigation of radiation damage needs multiscale approach, which concerns detailed knowledge of physical, chemical and biological phenomena following irradiation [1].

The most sensitive and reliable biomarkers for estimating radiation exposure, vulnerability to carcinogenesis or genomic instability are chromosome aberrations in peripheral blood lymphocytes [2–11]. The critical target to ionizing radiation in living cells is DNA. Since chromosomes are formed in the DNA condensation process during the mitosis of cell division, the chromosome aberrations (visible under light microscope) represent misrepair of DNA damage. The classical metaphase method, however, does not provide the whole spectrum of aberrations. Solid staining enables to detect structural aberrations like dicentrics, acentric fragments or centric rings, nevertheless symmetrical events like reciprocal translocations or complex exchange configurations are undetectable. In fact, no available experimental technique provides a high-resolution assessment of all types of chromosome [12]. Thus the statistical modeling of the data is much more informative than a simple comparison of average aberration yields.

The statistical analyses of biological processes based on the physical stages of ion track formations, which are already very well modeled using ab initio codes, have still many problems to include low-probability biological events and repair mechanisms. For this purpose, details of the track structure, clustering phenomena, and energy deposition distributions of different LET irradiations are of great importance not only for fundamental research but also for clinical applications. On the other hand, the importance of repair mechanisms can be investigated by analyzing the statistical distribution of aberrations among the cells at given dose [13]. Deviations from the expected Poisson distribution or the Neuman A distribution can provide indications about efficiency of the repair mechanisms [14, 15, 16]. This method, however, requires observation of rare multiple chromosome aberrations, which is difficult for low radiation doses.

In the present work, an alternative statistical method to assess the percentage of repaired chromosomes is considered. It is based on determination of the standard deviation of the individual damage factor of lymphocyte cells within a group of healthy persons or at one person but at different doses. The experiment was done either on peripheral lymphocytes exposed to gamma Co^{60} irradiation (LET equal to 0.350 keV/μm) or to 150 MeV proton irradiation (LET equal to 0.5 keV/μm).

2. Statistical effects of chromosome aberrations

In the ab initio models, the chromosome aberrations usually result from the ionizations processes of secondary electrons produced in the first stage of the interaction of the ion beam with a target material. The range of these electrons determines a radius of the ion track and the volume where the chromosome aberrations can take place. It is surprising that further evolution stages of the ion track, both physical and chemical, do not significantly contribute to the final probability of the DNA double strand breaks and chromosome occurrence. These effects obey the Poisson statistics, so finally we expect that the number of observed chromosome aberrations follows the Poisson statistics, or their convolution. However, it was recognized very early that the repair mechanisms can change this easy probabilistic scenario and cause some deviations from the Poisson statistics. The main effect known already for many decades is suppression of multiple chromosome aberrations in one metaphase

compared to the predicted Poisson distribution. For this purpose the Generalized Poisson Distribution (GPD) [16,17]can be applied by the following formula:

$$P_x(\tau,\theta) = \frac{\tau(\tau+\theta x)^{x-1}}{x!} e^{-\tau} \tag{1}$$

with the frequency of aberrations in a single cell: $x=0,1,2,3, \ldots$. The mean value is given by τ that is reduced by a negative value of the parameter θ. For $\theta = 0$ GPD takes the form of the usual Poisson distribution.

A new statistical effect we have recently observed in studies of chromosome aberrations is a reduction of the variance of the aberration number determined for blood samples of a group of healthy persons compared to the value expected for the Poisson statistics [18]. The same effect could be also found in the dose-effect curves measured for individual persons.

This kind of effects were demonstrated in physics of semiconductor detectors where the energy resolution of the detector determined by the variance of the energy loss process is much lower than that predicted by pure Poisson statistics. Reduction of the energy resolution described by the Fano factor arises from some energy loss processes, for instance atomic excitations that do not contribute to creation of charge carriers collected at the detector [19, 20]. Similar situation takes place in the processes responsible for production of the chromosome aberrations. Some of the DNA double strand breaks induced by ionizing radiation can be repaired (including dislocations) and cannot be observed under microscope as chromosome aberrations. In analogy to the Fano factor ,a repair factor RF can be defined as a ratio of the aberration number A' that would be observed in absence of any repair mechanisms and the aberration number A that is actually observed in the experiment:

$$RF = \frac{A'}{A} \geq 1 \tag{2}$$

Due to the above proportionality, the relative standard deviation during the repair mechanisms remains constant:

$$\frac{\sigma'_P}{A'} = \frac{\sqrt{A'}}{A'} = \frac{\sqrt{RF \cdot A}}{RF \cdot A} = \frac{1}{\sqrt{RF}} \cdot \frac{\sigma_P}{A} = \frac{\sigma_R}{A} \tag{3}$$

where the standard deviation σ_R is the real value observed in the experiment and corresponds to the theoretical one in absence of any repair mechanisms. The Poisson standard deviation $\sigma_P$is equal to the square root of the aberration number A. Thus, the repair factor RF can be now determined as follows:

$$RF = \frac{\sigma_P^2}{\sigma_R^2} \tag{4}$$

The corresponding percentage of repaired aberrations can be defined:

$$\frac{A'-A}{A'} \cdot 100\% = \frac{RF-1}{RF} \cdot 100\% \tag{5}$$

3. Materials and methods

Experiment with Co^{60} gamma rays was performed at the West Pomeranian Oncology Center, where a standard facility for Co^{60} radiotherapy was used. Proton irradiation and sample analysis was done in Joint Institute of Nuclear Research (JINR) in Dubna, Russia at JINR´s synchrophasotron (for more details see [17]).

Heparinized blood samples were obtained from healthy, young volunteers – males and females. Ethical approval was obtained for this study and all participants gave informed consent. The number of donors was: fifteen in the case of the gamma irradiation (at the dose 1 Gy/min for doses up to 2 Gy) and nine (getting 13 samples) for proton irradiation. The total dose used was 2 Gy with a dose rate of 1 Gy/min.

All activities were done in room temperature (21 °C).The culturing, fixation and slide making followed a standard procedure as described in IAEA (2001) Manual. For all patients at least two samples of diluted blood were prepared. Cultures were incubated at 37 °C. Cell division was stopped after 48 h by adding Colcemid. After an hour the cells were harvested and fixed by the routine hypotonic solution (0.56% KCl) and fixed three times with methanol/glacial acetic acid (5:1), mixed with the same amount of 0.9% NaCl.

Harvested lymphocytes were dropped on microscope slides and stained with Giemsa. All slides were coded and analyzed under light microscope. Types of aberrations considered were: dicentrics, centric rings, and acentric fragments.

For the purpose of statistical analysis, the individual damage factor (IDF) and average damage factor (ADF) were defined. IDF represents the ratio of aberrations (A) to all analyzed metaphases (X).

$$IDF = \frac{A}{X} \cdot 100\% \tag{6}$$

ADF represents the mean value and can be expressed as follows:

$$ADF = \frac{\sum_i A_i}{\sum_i X_i} \cdot 100\% \tag{7}$$

4. Experimental results and statistical analysis

The number of observed aberrations per 100 metaphases (*IDF* – individual damage factor) and the mean value (*ADF*) for samples exposed to 2 Gy of gamma and150 MeV proton irradiation are presented in Fig. 1 and Fig. 2 respectively. Assuming the Poisson statistics, the error bars of the *IDF* values corresponding to the 68% confidence level are simply equal to the square root of the aberration number (σ_P).

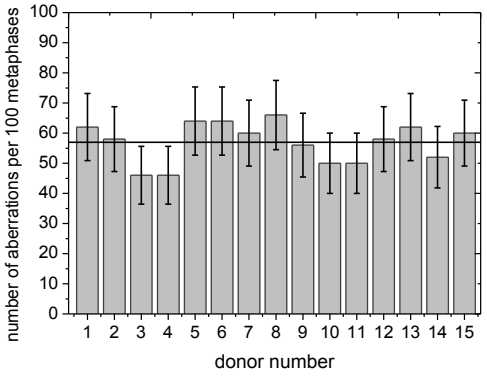

Fig. 1. Number of aberrations induced by 2 Gy gamma irradiation with the assessed ADF values; error bars represent uncertainties of aberration number counted according to the Poisson statistics

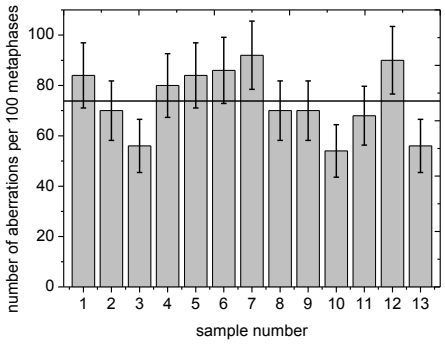

Fig. 2. Number of aberrations induced by 2 Gy proton irradiation with the assessed ADF values; error bars represent uncertainties of aberration number counted according to the Poisson statistics

As can be seen in Fig. 1 and Fig. 2, the IDF values are distributed very close to the mean values (ADF) and therefore it is to expect that the Poisson statistics overestimates the variance of experimental data. To estimate this effect, the real variance of the data collected can be calculated by the formula:

$$\sigma_R^2 = \frac{1}{n-1}\sum_{i=1}^{n}(IDF_i - ADF)^2 \tag{8}$$

and the average Poisson standard deviation of *n* samples:

$$\sigma_P = \frac{1}{n}\sum_{i=1}^{n} IDF_i \tag{9}$$

The RF values obtained for gamma and proton irradiation is presented in Table 1.

Table 1. Comparison of repair factors

	Gamma Irradiation	**Proton Irradiation**
Repair Factor (RF)	2.6 ± 0.3	1.19 ± 0.15
Percentage of Repaired Aberrations (%)	61 ± 4	16 ± 11

In Fig. 3 and Fig. 4, the distributions of chromosome aberrations determined in our study for all patients are compared to the pure Poisson distribution and to the generalized Poisson distribution (GPD) with the parameter θ fitted as a minimum of the corresponding chi-square value. The repair mechanisms can be recognized as underestimation of multiple chromosome aberrations for which the theta value is negative. The main disadvantage of the GDP method arises from the necessity for determination of a large number of multiple aberrations. Thus, it is mainly applicable for large dose studies. In the case of low dose investigations, they are very rare and therefore the uncertainty of the parameter θ is very high. As can be seen in Fig. 3 and Fig. 4, the overestimation of the multiple chromosome aberrations by the Poisson distribution is much larger for gamma than for the proton irradiation.

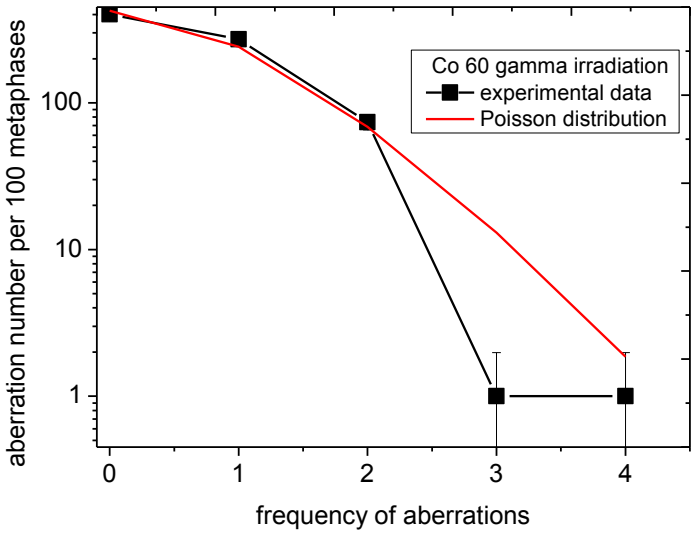

Fig. 3. Distribution of the chromosome aberrations induced by gamma irradiation obtained for all patients, compared with the Poisson distribution

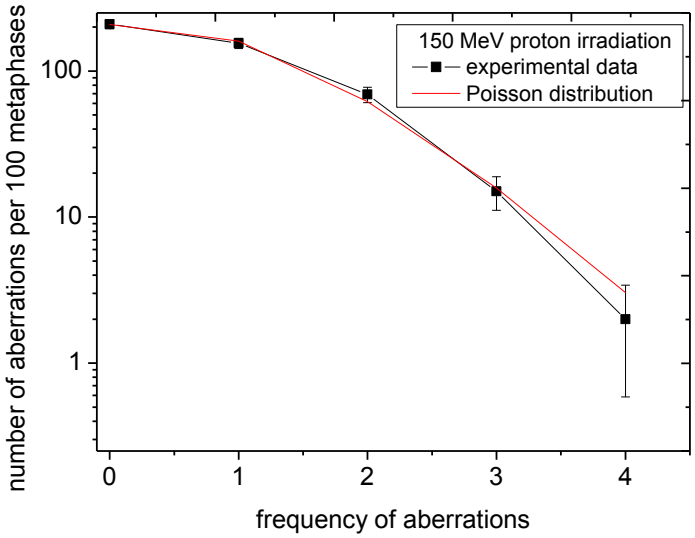

Fig. 4. Distribution of the chromosome aberrations induced by 150 MeV proton irradiation obtained for all patients, compared with the Poisson distribution

5. Discussion and conclusions

Study of statistical effects of chromosome aberrations can be a very powerful tool to estimate effectiveness of the repair mechanisms in individual cells. A reduction of the variance of the number of chromosome aberration in lymphocytes of the peripheral blood samples compared to that predicted by the Poisson statistics could be observed for gamma irradiation in the group of 15 volunteers for which about 60% of double strand breaks could be repaired. This founding agrees very well with the numbers obtained in investigations applying γ-H2AX [20]. The new method proposed seems to work much better at low doses for which generalized Poisson distribution analysis (GDP) shows strong limitations.

On the other hand, the repair factor obtained for protons ($RF = 1.19 \pm 0.15$) is very small in contradiction to our previous results obtained for the dose-effect curve [17] where RF was equal to

3.8 ± 0.8. This discrepancy indicates limitations of the new method. The small RF value determined in the present paper is probably consequence of three different persons that gauged the number of aberrations induced by the proton irradiation. As the individual uncertainties of judging persons could be larger than those resulting from the statistics, thus the dispersion of the IDF values around the ADF value could be artificially enlarged. In future studies, it should be ensured that the aberration analysis has to be performed only by one person.

The effects studied here are already known in other science fields where statistical methods are used. A very good example can be so-called Fano factor that describes reduction of the noise in the charged particle detectors leading to an increase of the detector energy resolution. This effect arises since the energy loss processes within the detector partially lead to an excitation of the detector crystal and not to creation of free charge carriers. The situation is very similar to reduction of the variance of the aberration number in the radiobiological studies. It can be visible only because some DNA double strand breaks can be repaired, resulting in properly-looking chromosomes. Analogously to the Fano factor for detectors, we observe under microscope, however, only that part of originally produced double strand breaks that can be recognized as chromosome aberrations.

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