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Individual Sensitivity of the Peripheral Blood Lymphocytes to the *in Vitro* Action of Mitomycin C in Children with Juvenile Rheumatoid Arthritis

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Abstract

The study was designed to estimate the levels of spontaneous and induced chromosome aberrations in the peripheral blood lymphocytes (PBL) of patients with juvenile rheumatoid arthritis (JRA) and their healthy peers. An increased spontaneous level of chromosomal aberrations has been found in children and adolescents with JRA in comparison with healthy age-mates. It has been established that the effect of the mitomycin C model mutagen on the PBL *in vitro* causes a multiple increase in the levels of chromosomal aberrations in both groups under investigation.

Keywords: chromosomal instability, cytogenetic analysis, juvenile rheumatoid arthritis, mutagenesis, peripheral blood lymphocytes, mitomycin C.

Introduction

A significant deterioration of the environment is being observed over the last years due to the latest achievements of scientific and technological progress, namely: the development of industry, the use of pesticides, herbicides and insecticides in agriculture, habitation of human beings in ecologically unfavorable areas, the use of drugs, bad habits, etc. [6]. All of these factors may have a negative impact on the human organism and cause disorders in the integrity of the genome. Undoubtedly, this is reflected on the function of the cell, its viability, i.e. on the availability of sound tissues and subsequently on the human health status. The probability of transformation of some aberrant cells into oncogenic cells has also been proved. It is widely accepted that chromosomal breaks and alterations, absence of chromosome disjunction in metaphase, endoreduplication and nuclear fusion form the basis of the initial stage of carcinogenesis [1, 8]. Therefore, it is necessary to conduct a combined assessment and monitoring of the cellular genome status in the people, belonging to the risk groups: the Chernobyl disaster liquidators and their descendants, workers of dangerous industrial branches, residents of contaminated areas, and patients with various multifactorial diseases. The most sensitive and simple method for bioindication of the mutagenic effect on living organisms is determining the spontaneous level of chromosomal aberrations. This method is recommended by the WHO and IAEA and is intended for the assessment of chromosomal and genomic disorders in the peripheral blood lymphocytes (PBL) *in vitro* [11].

To determine an individual hypersensitivity of a human being to the influence of mutagenic factors an additional mutagenic load on the PBL *in vitro* is used in the G2-phase of the cell cycle [16]. This method enables the authors to estimate a potential possibility of the genomic instability occurrence, when an organism gets in unfavorable environmental conditions [15]. Ionizing radiation is commonly used as a mutagen-provocateur, but over the last years specialists began to use chemical mutagens, and most of all bleomycin, antitumor antibiotic, which, being a radiomimetic, is able to induce a strong cytogenetic effect in the PBL *in vitro* [8, 15]. Some other mutagens, namely: dimatif [14], dimethoate, an insecticide, and mitomycin C, an antitumor antibiotic are used along with bleomycin to determine the sensitivity of the chromosomes to the effects of the chemical mutagenic load *in vitro* [4, 8].

Hence, determination of individual hypersensitivity of the PBL *in vitro* to a test-effect of mutagenic load is an extremely relevant and timely task. At present, the assessment of spontaneous and induced types of mutagenesis is carried out in various non-communicable diseases [7, 9]. Taking into consideration that a significant increase has been obtained in our previous studies in the level of spontaneous chromosome aberrations in probands with juvenile rheumatoid arthritis (JRA), in comparison with their healthy peers, the necessity to study the hidden chromosomal instability of the PBL *in vitro* arises in patients with JRA [3, 12].

Our study is aimed at determining the hypersensitivity of the PBL to the *in vitro* effect of mitomycin C model mutagen in children and adolescents with JRA.

Material and methods

Cytogenetic analysis was carried out in the State Institution “Institute for Children and Adolescents Health Care of the National Academy of Medical Sciences of Ukraine” in 30 children and adolescents of both sexes with JRA (main group) and 30 healthy children (control group), aged 5–17 years. To determine the sensitivity of chromosomes in patients with the JRA to the genotoxic effect of mitomycin C *in vitro* we have studied the levels of spontaneous and induced chromosome aberrations in chromosome preparations, obtained from the PBL culture.

The PBL cultivation was carried out according to the standard technique [19], for 72 hours at +37 °C, using PHA ("Sigma", Germany), RPMI 1640 medium and fetal calve serum. As a mutagen-provocateur we used mitomycin C, antitumor antibiotic, which was added to the culture at the 67-th hour of incubation at a final concentration of 3 μcg/ml. Stops of mitoses were performed at the 70-th hour of cultivation by addition of colchicine at the concentration of 0.1 μg/ml. After hypotonic treatment with KCl (0.075 M) for 12 minutes, the cells were fixed with a mixture of ethanol and glacial acetic acid (3 : 1). The cell suspension was pipetted on chilled wet slides, and ready preparations were dyed with Giemsa stain.

100 metaphase plates were analyzed to assess the frequency and types of chromosomal abnormalities. Disorders in the chromatid, chromosome and genome types were taken into consideration. Metaphase plates were examined using «Leica Galen III», «Ergoval» and «Leica CME» binocular microscopes, 10×18 eyepiece, 100×lens, and 1.25×binocular head.

Statistical calculations were performed on a PC. The coefficient of hidden chromosomal instability (C_{hci}) was calculated to reveal individuals with hypersensitivity to the action of mitomycin C [15]:

$$C_{hci} = M_{hci} / M,$$

where:

M_{hci} – individual values of the chromosomal aberrations frequency under the test-effect of mitomycin C at the concentration of 3 μg/ml;

M – average group values of the chromosomal aberrations frequency under the impact of mitomycin C at the same concentration.

According to Pilinskaya (2010), an induced cytogenetic effect exceeds the average group level of chromosomal aberrations in hypersensitive persons, so C_{hci} in them will always be > 1. The Student's t test was used to reveal the significance of differences between the compared parameters [2].

Results and Discussion

Average group level of spontaneous mutagenesis in the PBL of patients with JRA

corresponded to 4.35 per 100 cells and by 2.3 times exceeded the frequency of chromosomal abnormalities in their healthy peers (1.85 per 100 cells; $p < 0.001$). In the group of patients with JRA individual values of chromosomal instability ranged from 0 to 14 anomalies per 100 metaphases, moreover, the final values were recorded in isolated cases. In 16 patients (40 %) the level of chromosomal aberrations was within the limits of population norm (from 0.0 % to 3.0 %) [5]. Most frequent the individual values of spontaneous mutagenesis were within the limits of 4–6 disorders per 100 cells (45 % of the examined persons), which corresponded to the average finding in the group. A high level of chromosomal aberrations (from 7 to 14 per 100 cells) was revealed in 15 % of children and adolescents with JRA.

Addition of the mitomycin C model mutagen into the culture of the PBL resulted in a significant increase in the level of chromosomal aberrations in the main group (17.3, $p < 0.001$) and to 14.6 anomalies per 100 metaphase plates in the control group ($p < 0.001$).

Individual values of the induced mutagenesis in the study group ranged from 2 to 36 anomalies per 100 cells, and from 0 to 36 per 100 cells in the control group. To analyze individual hypersensitivity of chromosomes in the PBL *in vitro* in children and adolescents with JRA and in their healthy peers to the mutagenic load, we determined the difference between spontaneous and induced mutagenesis (supra-spontaneous cytogenetic effect) for each child. In both groups the distribution of supra-spontaneous level of chromosomal aberrations is approximately the same.

To determine the coefficient of hidden chromosomal instability (C_{hci}) the authors calculated the average group values of the chromosomal aberrations frequency (M) under the test-effect of mitomycin C. In the main group, this index came to 17.3 aberrations per 100 metaphases, and in the control group it was 14.6. In 14 patients (46.6 %) with JRA individual values of the induced mutagenesis exceeded the average group level of chromosomal abnormalities; in healthy age-matched children they were registered in 16 (53.3%) individuals. In addition, both groups were divided into two subgroups depending on the level of spontaneous chromosomal aberrations: main group ≤ 3.0 % (11 people) and ≥ 4.0 % (19 people); control group ≤ 3.0 % (22 people) and ≥ 4.0 % (8 people).

16.6 % of patients with JRA and normal levels of spontaneous mutagenesis had a hidden chromosomal instability. From 30 tested individuals 20.0 % had a low level of spontaneous mutagenesis, and they turned out to be resistant to the effects of the chemically-induced mutagenesis. An increased sensitivity of the PBL to the mutagenic load has been established in 33.3 % of probands with an elevated level of spontaneous mutagenesis (Fig. 1). The probands were most sensitive to the effects of mutagenes or they were debilitated patients with a high risk for the cells oncogenic transformation.

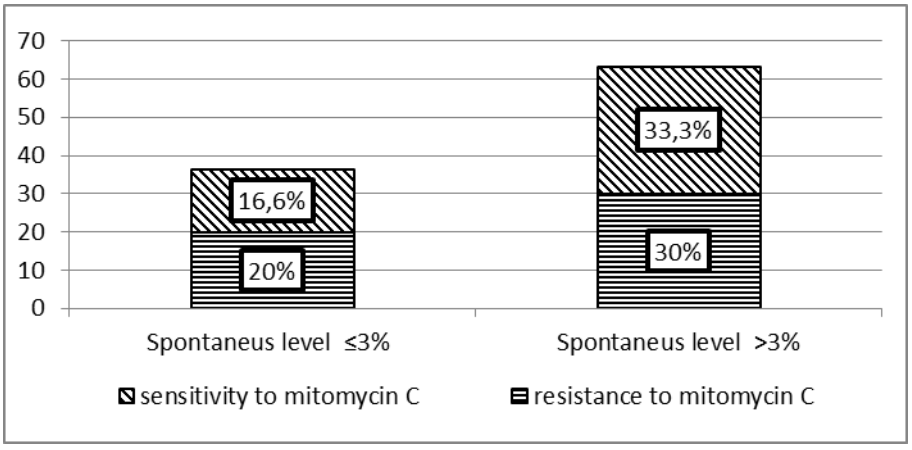

Figure 1. Distribution of sensitive and resistant to the mutagenic load patients with JRA and different levels of spontaneous mutagenesis

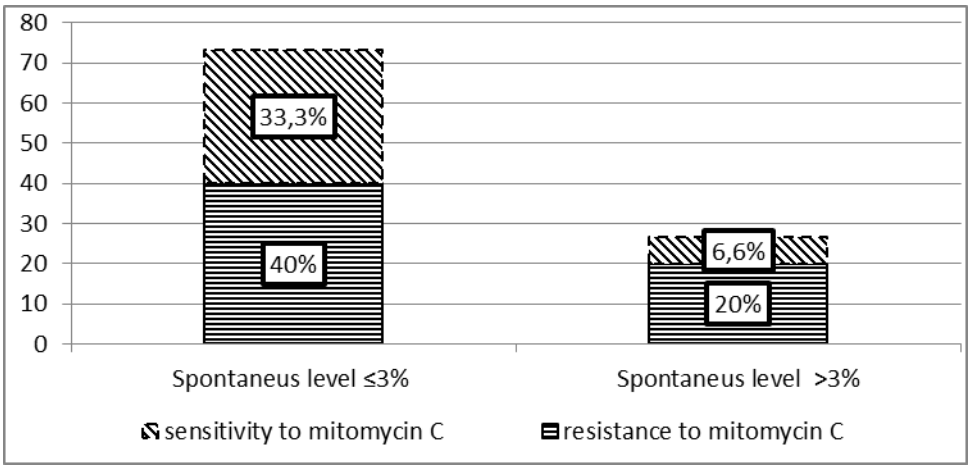

Figure 2. Distribution of sensitive and resistant to the mutagenic load persons among healthy peers with different levels of spontaneous mutagenesis

Sensitivity to the mutagenic load has been determined in 33.3 % of children from the control group with normal levels of spontaneous mutagenesis. In healthy children and adolescents with an elevated level of spontaneous mutagenesis the sensitivity of the PBL to the effects of model mutagen has been registered only in 6.6 % (Fig. 2).

Thus, we have determined an increase in the average group level of chromosomal aberrations in children and adolescents with JRA as compared with healthy peers. Analysis of the individual values of spontaneous mutagenesis in the represented groups has revealed the prevalence of individuals with an elevated level of chromosomal abnormalities in the main group. Moreover, the impact of the mitomycin C model mutagen on the PBL *in vitro* causes a multiple increase in the chromosomal abnormalities in the groups under investigation.

According to the data of literature [16], obtained at the determination of chromosomal radiosensitivity in healthy subjects, no relationship has been revealed between the individual findings of spontaneous mutagenesis and the value of the PBL individual radiosensitivity *in vitro*. The phenomenon testifies to the fact that not all the persons with high levels of spontaneous mutagenesis have an increased PBL sensitivity to the effects of the mutagen *in vitro*. The results obtained are consistent with the published data, as an increased individual chromosomal sensibility to the effects of the model mutagen is observed both at the elevated and normal levels of spontaneous mutagenesis in the two groups in approximately equal proportions (45.0 % – patients with JRA and 40.0 % – healthy peers). The existence of predisposing and protective to the effects of mutagens genotypes has been established owing to the work of some researchers [10, 13]. Perhaps this is a cause of approximately the same average group values of the induced mutagenesis in both groups.

According to our study, only 10 patients (33.3 %) among 30 children and adolescents with JRA have the increased levels of spontaneous and induced chromosomal abnormalities. These patients have the greatest risk of the pathological process complications and require constant monitoring of the chromosome apparatus state, as well as full control over the use of dietary or pharmacological antimutagens [6, 17, 18].

Conclusion

A multiple increase in chromosomal abnormalities has been established in the groups under investigation after addition of the mitomycin C model mutagen in the PBL culture. Distribution of supra-spontaneous levels of chromosomal aberrations is approximately the same in both groups which may be caused by the existence of the predisposing and protective genotypes, regarding the effects of mutagens.

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References:

1. Andreev S.G., Adelman Y.A. Ways exchange interactions damage leading to intrachromosomal aberrations depending on the structure of interphase chromosomes. *Radiation Biology. Radioecology* 41 (2001) 469-474.

2. Atramentova L.A., Utevskaya O.M. Statistical Methods in Biology. Gorlovka: Vidavnitstvo Lihtar. (2008) 248 p.

3. Bagatska N.V., Nefidova V.E., Medzianovska O.V. Evaluation of spontaneous and induced mutagenesis in peripheral blood lymphocytes of patients with juvenile rheumatoid arthritis. *Factors in experimental evolution of organisms* 10 (2012) 272-276.

4. Boltina IV, Kravchouk OP, Glavatsky OYa Chromosomal aberration in peripheral blood lymphocytes of patients with glial tumors in encephalon. *Oncology* 3 (2001) 23-25.

5. Boltina IV The use of the parameter «frequency of chromosome aberrations» in formation of risk groups regarding oncological diseases. *Cytology and Genetics* 41 (2007) 66-74.

6. Dvornic AS, Pererva TP, Kunakh VA Antimutagenesis as protection system of an organism against damaging factors of endogenous and exogenous origin. *Cytology and Genetics* 38 (2004) 62-71.

7. Gainetdinova D.D., Gaisina L.Z. Phenomenon of cell genome instability in the pathogenesis of children cerebral palsy. *Journal of Neurology and Psychiatry* 112 (2012) 49-51.

8. Hsu TC, Johnston DA, Cherry LM Sensitivity to genotoxic effects of bleomycin in humans: Possible relationship to environmental carcinogenesis. *International J. of Cancer* 43 (2006) 403-409.

9. Kinne R.W., Liehr T, Beensen V, Kunisch E, Zimmermann T, Holland H, Pfeiffer R, Stahl HD, Lungershausen W, Hein G, Roth A, Emmrich F, Claussen U and Froster UG Mosaic chromosomal aberrations in synovial fibroblasts of patients with rheumatoid arthritis, osteoarthritis, and other inflammatory joint diseases. *Arthritis Res* 5 (2001) 319-330.

10. Lin J, Swan GE, Shields PG, Benowitz NL, Gu J, Amos CI, Andrade M, Spitz MR and Wu X Mutagen sensitivity and genetic variants in nucleotide excision repair pathway: genotype-phenotype correlation. *Cancer Research* 16 (2007) 89-96.

11. Maznik NA, Vinnikov VA The dynamics of the cytogenetic effects in the peripheral blood lymphocytes of those who worked in the cleanup of the aftermath of the accident at Chernobyl Atomic Electric Power Station. Cytology and Genetics 31 (1997) 41-47.

12. Medzianovska O.V. Determination of the level of spontaneous chromosome abnormalities of the children who are down with juvenile rheumatoid arthritis. *The Journal of V.N.Karazin Kharkiv National University* 1079 (2013) 76-80.

13. Oliveira AL, Rodrigues FF, Santos RE, Aoki T, Rocha MN, Longui CA, Melo MB GSTT1, GSTM1, and GSTP1 polymorphisms and chemotherapy response in locally advanced breast cancer. *Genet Mol Res* 9 (2010) 1045-1053.

14. Pilinskaya M.A., Dybskiy S.S., Pedan L.R. (1993). Modification by dimatyph of cytogenetic damages in blood lymphocytes of children exposed to constant low-rate irradiation. *Cytology and Genetics* 27: 87-90.

15. Pilinskaya M.A., Dybskiy S.S., Dybskaya Y.E., Pedan L.R. (2010). Radiation induced modification of human somatic cells chromosomes sensitivity to the testing mutagenic exposure of bleomycin. *Cytology and Genetics* 44: 58-64.

16. Ryabchenko N.M., Dyomina E.A. (2008). Significance of spontaneous and radiation induced levels of chromosomal aberrations in estimation of human individual radiation sensitivity. *Vavilov Society of Geneticists and Breeders of Ukraine* , 6, 125-130.

17. Sloczynska K., Powroznik B., Pekala E., Waszkielewicz A.M. (2014). Antimutagenic compounds and their possible mechanisms of action. *J Appl Genetics* 55, 273-285.

18. Zasukhina G.D., Vasilyeva I.M., Shagirova Zh.M., Mikhajlov V.F., Vedernikov A.I., Gromov S.P. and Alfimov M.V. (2011). The effect of natural and synthetic antimutagens in human radiosensitive cells differing in genetic polymorphism *Doklady Biological Sciences* 440, 306-308.

19. Zerova-Lubimova T.E., Gorovenko N.G. (2003). Cytogenetic methodological investigation of human chromosomes: method. instruction. P. Shupyk KMAPE. 23 p.

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Индивидуальная чувствительность лимфоцитов периферической крови детей, больных ювенильным ревматоидным артритом, к тестирующему действию митомицина С *in vitro*

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Аннотация. Изучили уровень спонтанных и индуцированных хромосомных нарушений в лимфоцитах периферической крови больных ювенильным ревматоидным артритом (ЮРА) и здоровых сверстников. Определили повышение спонтанного уровня хромосомных нарушений у детей и подростков, больных ЮРА, в сравнении со здоровыми сверстниками. Установлено, что воздействие модельного мутагена митомицина С на ЛПК *in vitro* вызывает многократное повышение уровня хромосомных нарушений в обеих исследуемых группах.

Ключевые слова: хромосомная нестабильность, цитогенетический анализ, ювенильный ревматоидный артрит, мутагенез, лимфоциты периферической крови, митомицин С.