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The Results of Cytogenetic Examination of Bone Marrow under Irradiation in the Experiment

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Abstract

The distribution of the human or animal body varies the timing and causes of their death. The most common is the bone marrow form of acute radiation sickness, while depending on the type of mammals, death occurs on 7-30 days from the moment of irradiation, and the causes of death are most often hemorrhagic syndrome or infectious complications [3, 10, 11,13].

Electromagnetic oscillations with a small wavelength, X-rays and gamma radiation, streams of α- and β-particles (electrons), protons, positrons, neutrons and other charged particles, α-radiation and X-ray radiation have a high penetrating power, β-radiation has a lower penetrating power [5,15,17]. Radioactive substances can enter the body through intact skin, gastrointestinal tract, respiratory organs. After that, they are carried by the blood and lymph current to organs and tissues [3, 9, 12].

The body is most susceptible to the effects of radiation, especially for bone marrow cells. Under the influence of radiation, bone marrow aplasia develops, inhibition of mitotic processes in the organs of hematopoiesis, total death of low-grade bone marrow cells. A decrease in hematopoiesis is accompanied by the occurrence of hemorrhagic syndrome[2, 5, 8,14,16].

Chronic radiation sickness is a complex clinical syndrome that develops in the case of prolonged exposure to ionizing radiation in doses that exceed the permissible.Characteristic manifestations: duration and undulation of the course; the presence in clinical symptoms of both signs of damage to the body from the effects of radiation, and manifestations of restorative and adaptive reactions.Periods of development of chronic radiation sickness: the period of formation, or actually chronic radiation sickness; the recovery period; the period of the consequences of radiation sickness [4, 7,18].

The purpose of the study cytogenetic changes in bone marrow cells of white mongrel rats under chronic and acute irradiation were studied and evaluated in an experiment in a comparative aspect.

Materials and methods of research. To carry out the planned studies, 30 white mongrel male rats

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weighing 150-180 g were used, kept in standard vivarium conditions (room temperature 21-220C, relative humidity 50-60%, light mode - 12 hours of darkness and light). The maintenance of laboratory animals, feeding and caring for them, selection of animals, cleaning and disinfection of the vivarium premises were carried out according to Nuraliev N.A. et al. [6,12,16,18].

All laboratory animals (white mongrel rats) were obtained from the same nursery and of the same age. Before the start of experimental studies, all laboratory animals were kept in quarantine for 21 days.When working with experimental animals, all ethical principles of working with laboratory animals and rules of biological safety were strictly observed [1, 6].

All laboratory animals were divided into the following groups:

The first group consisted of white mongrel rats (n=12) who received acute radiation once at a dose of 5 Gray;

The second group consisted of white mongrel rats (n=12) who received chronic radiation for 20 days at 0.2 Gray daily;

The third group - intact white mongrel rats (n=6), who did not receive acute and chronic radiation.

During cytogenetic studies, all work with growth media and preparations was carried out in sterile conditions using a laminar box. Buffers were prepared on bidistilled water, filtered through membrane filters (0.22 microns "Millipor", Germany) and autoclaved at 1.2 atm. 30 minutes. The glassware is pre-sterilized at 1600C for 120 minutes before use. Equipment, fixtures, dishes made of polymer materials were exposed to ultraviolet light for 30 minutes. For experimental studies, bone marrow was selected from the femur of white mongrel rats during the autopsy of the animal.

Cytogenetic changes in rat bone marrow cells were studied using a direct method. The execution of the method included the following steps:bone marrow was washed out of the femur of white mongrel rats involved in the experiment of all three study groups with RPMI 1640 nutrient medium with 0.04% colchicine (which destroys the division spindle and chromosomes do not diverge to the poles during mitosis, forming a polyploid organism) into a centrifuge tube and incubated for 2-2.5 hours in a thermostat at 370C; incubated with hypotonic solution of CSl for 40 minutes in a thermostat at 370C; after hypotonization, the fixative was treated three times in the proportion of one part of glacial acetic acid and three parts of 96-1000 ethyl alcohol; the resulting precipitate was applied to a pre-cleaned degreased slide and stained with Giemsa dye; the search for metaphases was carried out under the microscope "Leica" (Germany) at 200 times magnification, the analysis of metaphase plates at 1000 times magnification, from 15 to 25 cells with metaphase plates were analyzed in each sample.

Statistical processing was carried out by generally accepted methods of variation statistics using programs for statistical analysis of biomedical research. The significance level of the indicator of the

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reliability of differences was considered P<0.05. When organizing and conducting research, the principles of evidence-based medicine were observed.

Research results and their discussion. For the analysis, we used bone marrow cells of laboratory animals that received and did not receive different types of radiation, in which elements of the mitotic apparatus were detected.

In the first group (acute irradiation), out of 123 examined bone marrow cells of laboratory animals, 72.36% (n=89) of the cells revealed normal metaphase plates, 12.19% (n=15) of the cells were at the prophase stage. It should be emphasized that 5.69% (n=7) of cells had polyploid cells (polyploidy), 9.76% (n=12) of cells had premature chromosome condensation.

Metaphase plates are a cluster of chromosomes in the plane perpendicular to the axis of division (the equatorial plane), in which the chromosomes are located equatorially in the metaphase of mitosis (the second phase of somatic cell division). The number of chromosomes in rats is normally 42 (diploid set).

Thus, the low content of cells (9.76%) with premature condensation of chromosomes and the absence of cells with pulverization and scattering of chromosomes indicates minor changes in the mitotic division of bone marrow cells of laboratory animals of this study group. The absence of animals in this group with low cellularity and low blast transformation (8.3%, n=1) indicates normal mitotic activity of bone marrow cells in all (n=12) laboratory animals. There is no pathology of mitosis in their bone marrow cells. Apparently, this fact is explained by the short observation period (5 days) of animals after a single acute irradiation, since it is believed, depending on the type of mammals, death occurs on 7-30 days from the moment of irradiation [2, 5].

Studies have proved that after a single acute irradiation (5 Gray) during the first 5 days, there are practically no changes in the mitotic division of bone marrow cells, chromosomal aberrations do not appear, mitotic activity does not decrease.

Further, the same studies were carried out with white mongrel rats that received chronic radiation (the second group).

Of the 125 bone marrow cells studied in laboratory animals of the second group, normal metaphase plates were found in 48.0% (n=60) cells, the prophase stage was observed in 8.80% (n=11) cells, in 2.40% (n=3) polyploid cells were found in cases, in 40.80% (n=51) cells cells with premature condensation of chromosomes were observed.

Of the 12 animals of the second group, 1 rat (8.33%) did not have mitotically dividing cells on the preparations, low cell count, low blast transformation and inhibition of mitosis were observed. The presence of cells with pulverized chromosomes indicates the pathology of mitosis.

Volume: 2 Issue: 8 | Aug – 2023 **ISSN: 2720-6866**
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The presence of a high concentration of cells (40.80%) with premature condensation of chromosomes in the bone marrow cells of rats of the second group indicates the inhibition of the normal mitotic cycle, which affects the proliferative activity of this tissue and the presence of cell clones with genetic pathology.

Unlike laboratory animals of the first and second groups, which underwent acute and chronic irradiation, in the bone marrow cells of white mongrel rats of the third group (intact) changes in bone marrow cells and the course of cell division were not observed, in all cases a normal karyotype was found - late metaphase.

Thus, in laboratory animals after acute single irradiation, the severity of cytogenetic changes was less pronounced than with chronic irradiation. There were no deviations from normal processes in intact animals.

Based on the conducted studies, cytogenetic changes in the bone marrow cells of laboratory animals receiving acute and chronic irradiation were studied and evaluated. The obtained data allow us to use the proposed recommendations to improve the effectiveness of the methodology for studying and evaluating cytogenetic changes in bone marrow cells of laboratory animals in experimental studies to determine the effect of different doses of radiation on the body.

Conclusions.

1. In the first group (acute irradiation), out of 123 examined bone marrow cells of laboratory animals, normal metaphase plates were detected in 72.36% of cells, 12.19% of cells were at the prophase stage. It should be emphasized that 5.69% of cells were polyploid (polyploidy), 9.76% of cells had premature condensation of chromosomes.

2. The low content of cells (9.76%) with premature condensation of chromosomes and the absence of cells with pulverization and scattering of chromosomes indicates minor changes in the mitotic division of bone marrow cells of laboratory animals of the first study group. The absence of animals with low cellularity and low blast transformation (8.3%) indicates normal mitotic activity of bone marrow cells in all laboratory animals. Apparently, this is due to the short observation period (5 days) of animals after acute irradiation, since it is believed that, depending on the type of mammals, death occurs on 7-30 days from the moment of irradiation.

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Volume: 2 Issue: 8 | Aug – 2023 **ISSN: 2720-6866**
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