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Abstract. The article contains the results of hematological, immunological and chemiluminescent analysis of the functional activity of peripheral blood cells of five-year-old lactating cows from the central regions of the Krasnoyarsk Territory (Siberia, Russia) with different radiation status, at which the radiation doses of animals were 0.02, 0.17 and 0.21 mSv per year. It was established that small doses of radiation at 0.17 mSv per year and 0.21 mSv per year affect hematological parameters, increase the phagocytic activity of blood leukocytes and change the kinetics of the formation of active oxygen forms by blood cells, which is manifested as an increase in the time of formation of the maximum kinetics of spontaneous and activated secondary radicals, reduction of the total production of secondary radicals in the antigenic activation of blood cells in vitro and an increase in the volume of spontaneously produced primary radicals.

1. Introduction

Radioactive emission is included into the list of factors that have a negative effect on biological objects, including animals and humans. On the territory of the Russian Federation, including Krasnoyarsk Territory, there are regions with a tense radiation situation, which is determined by the location of radiation-hazardous objects. In most parts of the Krasnoyarsk Territory, the radiation situation is assessed as favourable. Focal, point contamination of the floodplain of the Yenisei river with anthropogenic radionuclides is caused as a result of the discharge of cooling water flow of the reactors of the Federal State Unitary Enterprise "Mining and Chemical Plant" (FSUE MCP) [1].

Ionizing radiation in small doses acts on hematopoietic tissue, changes the biochemical parameters of blood of farm animals [2]. In cows kept under conditions of chronic low-dose irradiation (24-96 μR/hour), a significantly higher frequency of lymphocytic blood cells with micronuclei was detected in comparison with animals from clean zones (8-16 μR/hour) [3], as well as an increase in phagocytic peripheral blood neutrophil index [4]. Studies with laboratory animals have shown that the effect of low doses of ionizing radiation causes numerous structural changes in cells that persist for a long time after exposure and lead to changes in the functional activity of cells, including the activity of antioxidant enzymes, the rate of anion radical formation, and the composition and antioxidant activity of cell membrane lipids. [5]. A bimodal dependence of the change in the parameters studied on the dose value was found. Low-dose ionizing radiation of 0.48-33 mGy negatively affects the state of the

antioxidant system, causing changes in the state of erythrocyte membranes, their persistence [6], doses of 1-50 cGy lead to a significant change in the microviscosity of lymphocyte membranes [7]. It has been established that the processes of ROS generation by immunocompetent cells of the body are sensitive to the effects of even low concentrations of radionuclides and low doses of ionizing radiation [8-9]. But there are experimental data on laboratory animals indicating the activation of the immunogenesis system under the action of low doses of ionizing radiation and an increase in the body's resistance to higher doses [10]. Due to the lack of knowledge about the mechanisms of action of low-intensity radiation, there are difficulties in predicting the results of these effects on organisms and overcoming their potential negative consequences. Therefore, the study of the mechanisms of such effects is relevant both from fundamental and applied points of view.

The impact of ultra-low doses of radiation on the body can be assessed at the molecular level by chemiluminescent analysis. In this regard, the purpose of these studies was to determine the kinetics of the generation of reactive oxygen species by the cells of the peripheral blood of cows when exposed to ultra-low doses of ionizing radiation.

2. Materials and methods

Radioecological studies were conducted in accordance with regulatory documents on hay-pasture plots belonging to owners of cattle living in three settlements of the Krasnoyarsk Territory, having different radioecological status - the village of Borsk, the village of Bolshoi Baltschug and the village of Momotovo in the period 2016-2018. The village of Borsk is located 50 km to the north of Krasnoyarsk city, the soil and water of this territory do not have additional man-made and natural radiation pollution. The villages of Bolshoy Baltschug and Momotovo belong to FSUE MCP observation zone. The village of Big Baltschug is located on the right bank of the Yenisei river, 6 km from the border of the FSUE MCP sanitary protection zone. The village of Momotovo is located at a distance of 183 km to the north of Krasnoyarsk, on the right bank of the Yenisei river. Areas with additional man-made pressure, which was formed as a result of the activities of the FSUE MCP were identified on pasture lands of these settlements located on the bank of the Yenisei river.

Doses of cattle exposure were calculated according to EP 13.73.13/12-00 "Estimation of doses of irradiation of farm animals on the territory contaminated with radionuclides". Calculation of radiation doses was carried out on the basis of the data of radioecological examination of agrobiocenoses and values of specific activity of 137Cs in feed. It was established that the total dose of anthropogenic impact (external and internal exposure) on cows in B. Baltschug village was 0.21 mSv/year, in Momotovo village - 0.17 mSv/year, in Borsk town - 0.02 mSv/year. In Borsk, the dose of anthropogenic impact was within the limits of the values characterizing the global anthropogenic background, which is recorded throughout the region. The value of the equivalent dose of anthropogenic impact on cows in B. Baltschug and Momotovo settlements refers to the range of ultra-low dose values, as recommended by the Scientific Committee on Atomic Energy at the UN.

Lactating cows (51 heads) aged 64.08 ± 9.3 months became the object of the research. Peripheral venous blood from the caudal vein was the material for the study. The work was performed at the Department of Internal Non-Communicable Diseases, Obstetrics and Physiology of Agricultural Animals of the Institute of Applied Biotechnology and Veterinary Medicine of Krasnoyarsk State Agrarian University and the International Scientific Centre for the Study of Extreme States of the Body at the Presidium of the FIC KSC SB RAS. Analysis of hematological parameters (the number of red blood cells, leukocytes, ESR) was performed according to generally accepted methods [11]. Registration of the kinetics of ROS generation by cells of non-fractionated cow blood was carried out by the chemiluminescent method according to the method of V M Zemskov in the modification of I Yu Eremina with coauthors [12] on the 36-channel "Chemiluminometer 3604– PEVM" hardware-software complex (SKTB "Nauka" SB RAS, Russia). To enhance chemiluminescence, chemiluminescent (CC) probes solutions of 0.1 mmol/l of lucigenin and 0.22 mmol/l of luminol in Hanks solution (pH 7.4) were used, which are selective for primary and secondary oxygen radicals,

respectively. The activation of blood cells was carried out in vitro with latex particles (FGUP NIISK, St. Petersburg) at a concentration of 5x108 ppm/ml, opsonized with cattle pulp serum proteins, after staining with 0.25% gentian violet in a 3% acetic acid solution. The recording time of the chemiluminescent curve is 180 minutes, the temperature in the recording chamber is + 38 ° C. The following parameters of the kinetics of ROS generation were investigated: the area under the chemiluminescent curve (S, imp. for 180 min), which characterizes the total amount of generated radicals; activation index (IA = S_{act}/S_{spont}, usd.). Phagocytic activity of leukocytes in relation to latex particles was evaluated at the end of the chemiluminescent reaction.

Statistical processing of digital material was carried out by the variation statistics method - Student's t-test using Microsoft Office Excel 2007 application programs. The differences in the CC parameters were considered reliable at $P \leq 0.05$.

3. Results

Hematological analysis of the peripheral blood of cattle showed that the number of erythrocytes, leukocytes and ESR in animals in areas with different radiation hazards is in the same range of variability. However, when cows were exposed to doses of 0.17 and 0.21 mSv/year, there was a tendency to leukocytosis and erythrocytosis compared with data obtained with a dose of 0.02 mSv/year (table 1), which indicates the stimulating effect of ionizing radiation on hematopoiesis.

Table 1. Hematological parameters of cattle with different dose load.

Radiation dose, mSv/year	White blood cells, $\times 10^9$/л	Red blood cells, $\times 10^{12}$ /л	ESR, mm/h
0.02	7.71 ± 1.17	6.78 ± 0.81	0.99 ± 0.22
0.17	8.10 ± 0.43	9.26 ± 0.74	1.14 ± 0.21
0.21	9.15 ± 1.67	9.23 ± 1.24	0.65 ± 0.13

The study of the phagocytic activity of blood cells in relation to latex particle opsonized by proteins of the pool serum of cattle showed some dependence of the phagocytic index on the dose of exposure to ionizing radiation on the organism of lactating cows (figure 1). With an irradiation dose of 0.21 mSv/year, the indicator grew by more than 16% relative to the dose of 0.02 mSv/year.

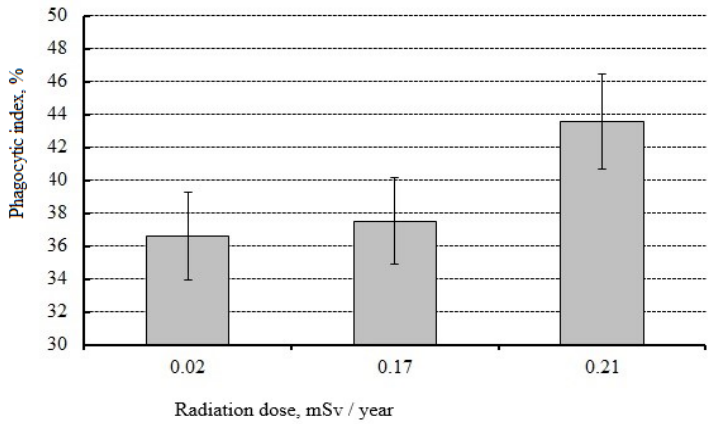
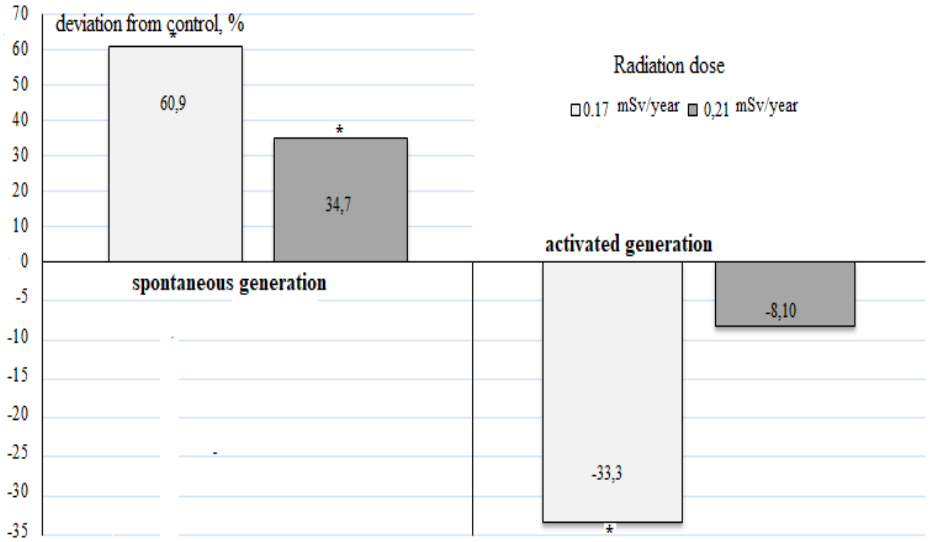


Figure 1. Phagocytic index of white blood cells in cattle in agrobiocenoses with different radiation intensity.

We have established that the chemiluminescent kinetics of the generation of primary lucigenin dependent and secondary luminol dependent radicals in bovine blood cells had specific features in animals with different doses of ionizing radiation.

The total volumes of primary radicals spontaneously generated in the blood of cows exposed to radiation doses of 0.17 mSv/year and 0.21 mSv/year significantly ($P < 0.05$) exceeded the blood levels of animals at a dose effect of 0.02 mSv/year (figure 2). The total production of lucigenin dependent

ROS in in vitro antigen activation was lower in animals exposed to doses of 0.17 mSv/year and 0.21 mSv/year, by 33.3% and 8.1%, respectively, compared to an exposure dose of 0.02 mSv/year.



Note: * P<0.05

Figure 2. Deviation from the reference light sum of lucigenin-enhanced chemiluminescence during spontaneous and antigen-activated in vitro production of ROS by cells in cattle blood at radiation doses above 0.02 mSv / year (control).

The total amount of luminol dependent radicals, spontaneously generated by blood cells of cows exposed to radiation in doses of 0.17 and 0.21 mSv/year, is lower by 46.7% and 17.1%, respectively, compared to animals irradiated at a dose of 0.02 mSv/year (figure 3). With in vitro antigen activation of cells in the blood of cattle exposed to radiation doses of 0.17 mSv/year and 0.21 mSv/year, the total amount of luminol-dependent radicals was significantly (P <0.01) lower than in animals that received radiation at a dose of 0.02 mSv/year

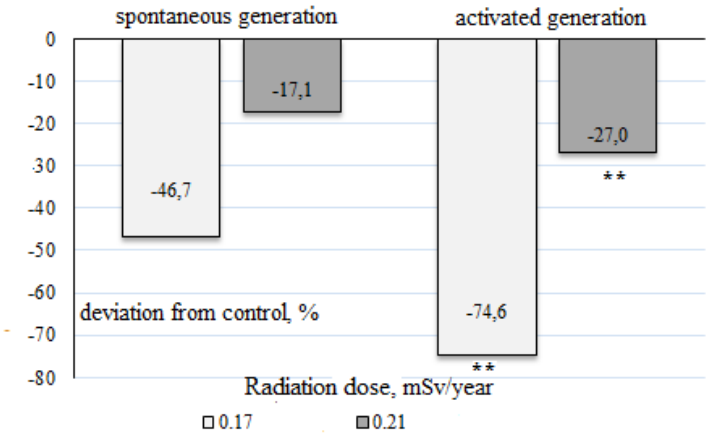


Figure 3. Deviation from the reference light sum of luminol-enhanced chemiluminescence during spontaneous and antigen-activated in vitro production of ROS by cells in cattle blood at radiation doses above 0.02 mSv/year (control).

Note: ** P<0.01

Based on the obtained data, we calculated the activation index (AI) of chemiluminescence, which reflects the ability of blood cells (neutrophils, monocytes) to generate ROS in response to antigenic stimulation in vitro (figure 4). The activation index for luminol and lucigenin dependent ROS in cattle was found to be 1.4 times less when exposed to an irradiation dose of 0.21 mSv/year than in animals at

a dose of 0.02 mSv/year, which indicated a decrease in the reserve capacity of neutrophils involved in non-specific resistance of the organism, to the generation of ROS.

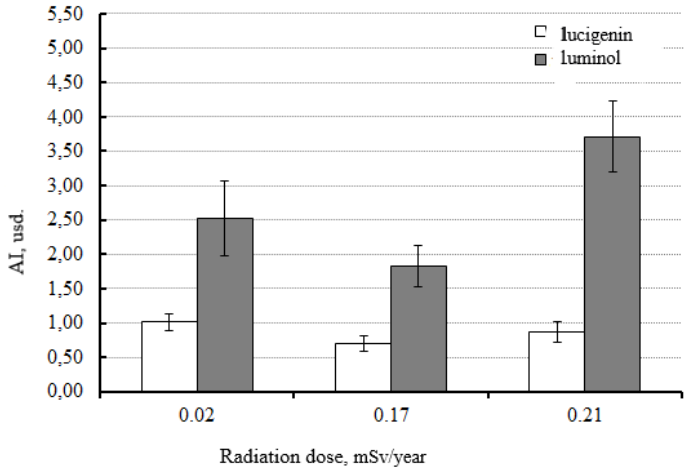


Figure 4. The value of the activation index (AI) of the generation of ROS by peripheral blood cells of cattle living in areas with different dose levels.

4. Discussion

Hematological indices and phagocytic activity of white blood cells in lactating cows contained in areas with a background and man-made radiation dose of 0.02 mSv/ ear, 0.17 mSv/year and 0.21 mSv/year did not differ significantly, indicating that the somatic health of animals. However, a tendency to an increase in the number of white and red blood cells, and phagocytic index has been found out especially when the dose of ionizing radiation is 0.21 mSv/year.

O S Izmestieva with co-authors revealed in 2014 that when small doses of ionizing radiation are exposed to laboratory animals, the activity of the antioxidant system of erythrocytes changes at a tenfold increase in the dose (4.8 mGy per 20 hours). A significant decrease in the activity of not only SOD, but also catalase was recorded, which persisted up to eight days after irradiation. The works of M Yu Alesina (1999) showed that chronic exposure to low-intensity γ-radiation at a dose of 8 cGy in mice caused a noticeable change in red blood cells, manifested in a decrease in the antioxidant status of lipids even in long periods (7 months) after irradiation [9]. Short-term exposure to low-intensity ionizing radiation in a dose of 1 to 10 cGy, according to L N Shishkina with co-authors (2015), activates the immune system [12], but prolonged exposure slows down the differentiation and maturation of hematopoiesis cells [8].

We found that under the influence of low doses of radiation - 0.17 and 0.21 mSv/year, in lactating cows living under the action of background irradiation dose values of 0.02 mSv/year, the kinetics of ROS generation by blood cells changed. The total number of spontaneously generated primary radicals in the blood of cows when exposed to an irradiation dose of 0.17 mSv/year and 0.21 mSv/year was significantly ($P < 0.05$) higher, and the secondary antigen-activated in vitro was significantly ($P < 0.01$) lower than that of cows under the action radiation doses of 0.02 mSv/year.

The activation index of luminol and lucigenin dependent ROS generation in in vitro antigen activation in cows kept in a territory with a dose load of 0.21 mSv/year is 1.4 times less than in animals of the control background zone (0.02 mSv/year), which indicated a decrease in functional neutrophil potential. An increase in AI with the generation of secondary radicals in cows at an irradiation dose of 0.21 mSv/year may be associated with a sharp increase in the production of lipid peroxide radicals, or with the presence of "immature" neutrophils [13-14], or insufficient activity of the antioxidant system of cells and plasma, under the which is the formation of lipid peroxides.

5. Conclusion

Thus, the effect of low doses of ionizing irradiation of 0.17 mSv/year and 0.21 mSv/year on the organism of lactating cows slightly stimulates hematopoiesis, enhances the phagocytic activity of

white blood cells and changes the kinetics of generation of active oxygen forms that play an important role in antimicrobial and antitumor immunity.

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