An impact of low doses radiation on the kinetics of reactive oxygen species generation in sheep peripheral blood

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Abstract. The work is devoted to measuring the impact of low doses of ionizing radiation on the kinetics of reactive oxygen species (ROS) generation in sheep peripheral blood. In the evaluation of the OAF production intensity level by the peripheral blood cells in the animals existing on the territories with different radiation tense by chemiluminescent method, it was established that having increased dose tense from 0,91 till 1.55 mGy/ year, the amplitude of maximum intensity credibly increased, as well as the total amount of spontaneous and antigen activated primary lucigenin dependent ROS production and antigen activated secondary luminol dependent ROS with time decrease for the maximum achievement of the respiratory explosion.

1. Introduction

Biological objects are constantly under nature radiation background's impact and suffer human impact within restricted areas. Annual absorbed dose of radiation consists of external and internal impact of ionizing radiation. The external radiation from natural sources is formed under the influence of cosmic radiation and gamma-mapping from the natural radionuclides of the rock. The internal radiation is a flow of natural radionuclides with the ration, water and air. The pollution of the land with technogenic radionuclides adds value to annual absorbed dose, increasing it. The research of the impact of low doses of ionizing radiation on biological objects is relevant nowadays.

There are territories in the Russian Federation with intense radiation situation, it's determined by the placement of the radiation objects, technogenic radionuclides content in soils from the earlier times, and Krasnoyarsk region is related to such territories. In most of Krasnoyarsk region radiological situation is evaluated as successful [1]. On its territory a point pollution of the river Enisey floodplain with technogenic radionuclides was found. It was a result of water discharge from the cooling of stream reactor of the Federal State Unitary Enterprise "Mining and Chemical Plant" (FSUE MCP) which is situated in Zheleznogorsk town [2, 3]. In 2006 a monitoring area was established for FSUE MCP, covering 20 km around the enterprise and 1000 km downstream of the Enisey floodplain from Zheleznogorsk. Around the 20th km of the monitoring area of FSUE MCP there are 12 rural population centres and Zheleznogorsk town itself. There are more than 30 population centres around
the 1000th km in the Enisey floodplain observing area. The point pollution in the area of the Enisey floodplain is from The Mining and Chemical Plant to the Kara sea. It is characterized by spotting and different components of radionuclides [4, 5].

The understanding about the reactions of biological systems on the impact of low doses ionizing radiation has been changed recently. Low doses change cells and tissues processes, resulting in change of functional activity of cells and tissues [6]. The usage of the chemiluminescent method for the evaluation of the animals physiological conditions used Serkis Y. I [7] for the first time. Nowadays chemiluminescent methods are widely used for monitoring antioxidant and pro oxidant properties of biological systems [8, 9, 10].

It has been established that generation processes of the reactive oxygen species by the organism cells are susceptible to the impact even of the lowest doses of ionizing radiation [11]. The research of the impact mechanism of low doses radiation on the farm animals' organism is a prospective, fundamental and applied direction. Highly sensitive chemiluminescent methods reflect the state of free-radical processes making possible to identify their minor changes under different physiological conditions. The research about changes evaluation of the chemiluminescent kinetics of the reactive oxygen species generation by the sheep's peripheral blood cells under the impact of low doses radiation in combination with the data of radiological examination of agro-ecosystems has not been held yet. A detecting of impact measuring of radiation on the kinetics of the oxygen active forms generation in sheep's peripheral blood under the condition of Krasnoyarsk region agro-ecosystems with a different dose of radiation is relevant and meaningful. The results of the work will be relevant for the regions of the Russian Federation with additional technogenic pressure and for the countries with point pollution territories by technogenic radionuclides.

2. Materials and methods
The research was held according to the regulations during the period 2016-2018 in agro-ecosystems of Sukhobuzimsky region of the Krasnoyarsk territory in B.Balchug and Borsk villages.

Bolshoy Balchug village is situated above the floodplain area of the right bank of the river Enisey channel, it's 6 km away from the border of the FSUE MCP health protection zone. On the pasturelands, situated on the Enisey river bank, places with additional anthropogenic load were determined, which were the results of the earlier activity of FSUE MCP. Borsk village is situated 50 km north from Krasnoyarsk and it is a reference place, soils and water of which do not have additional technogenic and nature radiation pollution.

In the period from 2016 till 2018 some tests for measuring gamma-mapping were held by means of the search radiometer «SRP-68-01» and multifunctional wide spectrum professional dosimeter «DRG-01T1». On the territory of agro-ecosystems soil samples were taken by the gamma spectrum method and it was determined that they have a specific activity of technogenic radionuclides in the geometry of Marinel vessel during 3600s on the gamma spectrometers «01 Radek» and «Gamma-1C»

For the chemiluminescent analysis during spring and autumn veterinary examinations some blood tests from 30 small cattle were taken. The evaluation of the kinetics of the reactive oxygen species generation was done by chemiluminescent method. Lucigenin (Sigma-Aldrich) and luminol (Sigma-Aldrich) were used as ChL-probes. The following indicators were determined: maximum activity amplitude (I_max, impulse/sec); maximum reached time (T_max, min); light sum (S, impulse/90min); activation index (AI=S act/S spont, common unit). Chemiluminescent analysis was held under Zemskov V.M. method by co-authors [12] in our modification [13]. The kinetics of spontaneous and antigen induced chemiluminescence was recorded on 36-chanel hardware and software complex «Chemiluminescentmeter 3601-PEVM» (SCB «Science» SD RAS). The record time of the chemiluminescent curve is 90 min, the temperature in the registration cuvette + 38 C. Sheep's radiation doses were calculated according to Veterinary rules 13.73.13/12-00 «The evaluation of radiation doses of the farm animals on the territory polluted by radionuclides» The statistical processing of the digital material was carried by the variational statistic method with the help of Microsoft Office Excel 2007 applications. The differences in metrics ChL were considered credible under P≤0.05.
3. Results
During the research of the soils in B. Balchug village agro-ecosystems it was established that the average of specific activity $^{137}\text{Cs}$ was $166.69 \pm 33.09$ Bq/kg, whereas in Borsk village it was $13.27 \pm 1.30$ Bq/kg. The specific activity $^{137}\text{Cs}$ in B. Balchug soils is 12.6 times higher than in Borsk village (it was established with the confidence level $P < 0.001$). However, the value of dose rate of the external gamma-mapping in the agro-ecosystems of B.Balchug village does not exceed the value regulated by normative documents of both federal and regional levels [14].

According to the regional standard, the capacity of gamma-mapping dose in agro-ecosystems of B. Balchug village is $0.18$ µGy/h, it is lower than the registration level, established by regional standards of the environment quality. According to data provided by the specific activity $^{137}\text{Cs}$ in the agro-ecosystems of B. Balchug village and based on the regional standards of the environment quality in the radiation security area, a periodic control of radiation situation needs to be held [15].

Farm animals had similar composition of the ration, the difference was only in the concentration of $^{137}\text{Cs}$. The specific activity of $^{137}\text{Cs}$ in the grass of B.Balchug pasturelands was $54.0 \pm 3.5$ Bq/kg, in the grass of Borsk it was $14.9 \pm 2.3$ Bq/kg. The specific activity in the hay was $14.9 \pm 0.71$ Bq/kg in B.Balchug and $7.1 \pm 0.94$ Bq/kg in Borsk.

According to the veterinarian rules(VR) 13.73.13/12-00 the absorbed doses of external and internal radiation are used for the evaluation of the radiation impact level on the farm animals organisms. The value of annual absorbed dose of the farm animals external and internal radiation is presented in table 1.

| The components of absorbed dose, mGy | The population centre | B. Balchug village | Borsk village |
|-------------------------------------|----------------------|-------------------|---------------|
| The external radiation in the pasture period in daytime | 0.37 | 0.15 |
| The external radiation in the pasture period at night | 0.23 | 0.14 |
| The external radiation in the housing period | 0.94 | 0.62 |
| The internal radiation in the pasture period | 0.0062 | 0.0014 |
| The internal radiation in the housing period | 0.0069 | 0.0033 |
| The total annual absorbed dose | 1.55 | 0.91 |

The radiation dose in these population centres is different in the value of the external and internal radiation in the pasture period in daytime. The external radiation is determined by different gamma-mapping in the pasturelands (table1), the internal one is determined by different $^{137}\text{Cs}$ concentration in green fodder. As a result of radiation dose calculation, it was determined that the total impact dose (external and internal) on sheep's organism in B. Balchug village was 1.55 mGy/year, and in Borsk village it was 0.91 mSv/year. In Borsk village the value of technogenic impact dose is within the levels describing the global technogenic background which is registered in the territory of Krasnoyarsk region. The radiation dose of the farm animals in B. Balchug village is 1.7 times more than the dose in Borsk. The value of technogenic impact on the farm animals in B. Balchug village is related to the range of super-small doses value, according to the recommendation of the nuclear power scientific committee to the UN.

The kinetics of spontaneous and antigenactivated «in vitro» ROS generation by the peripheral blood cells of the sheep was determined by the chemiluminescent method. The time of creation peaks of primary lucigenin dependent reactive oxygen species (mostly superoxide anion $\text{O}_2^-$) in sheep's blood which are under the impact of low doses radiation 1.55 mGy/year and the activity of background values 0.91 mGy/year do not accurately differ. All the animals had maximum intensity of the peak of spontaneous production lucigenin dependent radicals in one range of variability and was registered for 29 min. After the phagocytosis activation with latex particles in the presence of lucigenin, the top of chemiluminescent curve was registered at 28 min on average of all the animals, regardless of the
pollution density of the territory. Antigen activation of blood cells "in vitro" with latex particles potentiates the activation of pro oxidant reactions. The increase of the reactive oxygen species (ROS) quantity is the proof of the animals blood cells reactivity and organism ability to respond the antigen attack.

The peak of spontaneous generation activity of secondary luminol dependent reactive oxygen species (H$_2$O$_2$, OH, HClO) in animals blood with the radiation dose 0.91 mGy/year was registered at 29 min (28.77 ± 2.9 min). The secondary radicals among the sheep from the territories with radiation pressure (1.55 mGy/year) are formed credibly (P < 0.01) 9 min faster with Tmax 20 min on average (19.89 ± 1.3 min). After blood cells activation with the latex particles in the presence of luminol, the fastest top of chemiluminescent curve (24.22 ± 2.5 min) was registered in sheep blood with 1.55 mGy/year dose. The sheep which had a radiation dose 0.91 mGy/year, antigen activated explosion reached the maximum credibly (P < 0.01) 20.6 ± 2.04 min later.

The maximum amplitude (I$_{max}$, imp/s) of the spontaneous and activated primary radicals generation in animals blood, under the radiation dose 1.55 mGy/year, was credibly (P < 0.01) higher than in animals blood under the radiation dose 0.91 mGy/year (figure 1).

![Figure 1](image_url)

**Figure 1.** The value of I$_{max}$ lucigenin (a) and luminol (b) reinforced OAF chemiluminescent kinetics generation by the sheep blood cells under different radiation doses.

After the phagocytosis activation by latex particles, the maximum amplitude does not change with 0.91 mGy/year dose. Having added latex particles into the sheep peripheral blood from the territory with 1.55 mGy/year radiation dose, a tendency to the increase in the maximum amplitude of the primary ROS generation is determined. The activity of maximum amplitude (I$_{max}$, impulse/sec) of the spontaneous generation of secondary OAF among the animals with different radiation doses activity did not differ. The activation of blood cells by latex particles leads to the increase of secondary ROS generation (figure 2b). Among the animals with 1.55 mGy/year dose, a credible (P < 0.05) increase of maximum amplitude generation under the addition of the latex particles "in vitro" into peripheral blood is determined. The total quantity of primary radicals, spontaneously generated during the registration time in sheep's blood with 1.55 mGy/year dose, is credibly higher than among the animals with 0.91 mGy/year dose (figure 2). After latex particles addition, the total ROS production in all the animals blood increases.

The total quantity of the secondary ROS, spontaneously generated in sheep's blood with 1.55 and 0.91 mGy/year doses is within one range of variability and does not differ in statistics (figure 2). After the phagocytosis activation by addition "in vitro" of latex particles, the total ROS production in the all animals blood is increased.
Figure 2. The value of lucigenin and luminol reinforced chemiluminescence light sum with spontaneous and antigen activated "in vitro" ROS production by sheep blood cells under different radiation doses.

Based on the data received the activation index (AI) was calculated. It reflects the ability of blood cells (neutrophils and monocytes) to generate ROS against antigen stimulation (in vitro) (figure 3).

Figure 3. The value of the activation index (AI) of the sheep peripheral blood ROS sells generation under the impact of low radiation doses.

The activation index (AI) of luminol and lucigenin dependent ROS generation, among sheep under the effects of different absorbed doses is within one range of variability and does not differ in statistics. This emphasizes the equal spare capacities of neutrophils to the ROS generation. A slight tendency to the increase of the primary and secondary radicals activation index (AI) was determined among the sheep under 1.55 mGy/year radiation dose.

4. Conclusions

As a result it was established that the total quantity of spontaneous secondary radicals did not differ among the sheep with 1.55 mGy/year and the sheep with 0.91 mGy/year absorbed doses. Besides, the time for achieving maximum of spontaneous and activated formation of the top for the primary free radicals and spontaneous generation of secondary radicals was at 28 min. The activation index of luminol and lucigenin dependent ROS generation among the animals with 0.91 and 1.55mGy/year is close.

A slight tendency to the AI primary and secondary radicals increase was determined among the sheep with 1.55 mGy/year absorbed dose. An acceleration of antigen activated process formation of free radicals, an increase in the amplitude of the maximum in spontaneous and activated primary radicals generation and an increase of the total quantity of primary radicals production were established in the peripheral blood among the sheep with the total absorbed 1.55 mGy/year dose.
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