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The effect of separate and combined action of lead oxide and gamma radiation on immunological reactivity and biochemical processes in the organs of immunogenesis

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Abstract---Background: The problem of the effects of ionizing radiation on the human body in the conditions of professional activity has attracted increased interest in recent years. This article is devoted to the study of the results of the influence of negative environmental factors on a living organism in real conditions, which is almost always combined. Objective: to evaluate the effect of separate and combined exposure to low doses of ionizing gamma radiation and oral intake of lead (IV) oxide into the body on the activity of oxidative, antioxidant mechanisms and cellular immunity in the experiment.Materials and methods: Studies were conducted on 120 white mongrel sexually mature rats: 4 groups of equal numbers, males weighing 180-270 g. The state of lipid peroxidation and antioxidant protection was assessed in the tissues of the thymus and spleen. The state of antioxidant protection was assessed by the activity of catalase, glutathione reductase and glutathione peroxidase in the same tissues. Indicators characterizing the cellular link of immunity were determined in all experimental animals. Results and discussion: CD4+ content indicators were lower than in the control group by 24.7% under irradiation, by 18.9% under lead oxide priming and by 31.4% when combined (p=0.021, p=0.043 and p=0.015, respectively). A

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significant decrease in the number of CD8+ was determined in both groups of exposure to ionizing radiation, while in the group of combined influence of negative factors there were no differences from isolated gamma irradiation (differences with control by 24.3% and 25.7%, p=0.035; p=0.033). The index of inhibition of leukocyte migration during irradiation significantly decreased. The index of production of reactive oxygen species by phagocytic cells was significantly lower in the group of combined exposure. In terms of IL-1 content, a significant decrease was determined when experimental animals were primed with lead oxide and with combined exposure, the differences with the control were 19.4% and 26.1% (p=0.040 and p=0.019). Conclusions: Analysis of the state of antioxidant mechanisms allowed us to conclude that the factors used in the experiment had a significantly lower effect on their activity in thymus tissues. Thus, we identified only one parameter for which significant differences with the control were registered – the activity of GR in the group of combined exposure, which was 39.7% lower (p=0.025).

Keywords---lead oxide, gamma radiation, immunity, oxidant, antioxidant, cellular and humoral immunity.

Introduction

Research rationale, The influence of negative environmental factors on a living organism in real conditions is almost always combined. However, some of the negative impacts may reach high and extreme values at a particular time, while others may be background and have a gradual effect over a long period. In addition, a combination of several extreme factors is quite likely, especially in the conditions of natural and anthropogenic disasters [1,3].

Exposure to ionizing radiation, the level of which has increased as a result of the activities of human civilization in specific regions — this is the Semipalatinsk test site of the Republic of Kazakhstan, Hiroshima and Nagasaki, Japan, where public exposure is one of the main problems of human ecology in the nuclear era [2,4,6]. In modern conditions, the local production activity of nuclear power plants (Focusimo Japan) [36] creates local foci of additional irradiation, and they usually correspond to areas of high content of other pollutants in the air, soils and water [5].

One thing should be noted: the direction of the realization of the toxic effects of certain chemical compounds and ionizing radiation, realized through the activation of free radical oxidation in the cells and tissues of the animal body [7]. One of the targets of this effect may be the immune system, in particular, the process of formation and vital activity of lymphocytes [8,11].

The high relevance of this problem necessitates further targeted research in this direction. In this direction, the state of the cytokine profile under the separate and combined action of radiation and lead oxide is of great interest, since they have a close connection with all parts of the immune system.

Objective: To evaluate the effect of separate and combined exposure to small doses of ionizing gamma radiation and oral intake of lead (IV) oxide into the body on the activity of oxidative, antioxidant mechanisms and cellular immunity in the experiment.

Materials and methods

Type of study – a randomized controlled prospective study was conducted. All procedures on animals are coordinated and approved by the Ethics Committee of the State Medical University of Semey, Kazakhstan (Protocol No. 11 of 27.09.2017) in accordance with the directive of the European Parliament and the Council for the Protection of Animals.

The studies were carried out on 120 white mongrel sexually mature rats: males weighing 180-270 g.

During the study, they were in a specialized room for keeping animals in the vivarium of the Semey State Medical University, which complies with international rules and "Rules for conducting preclinical studies, biomedical experiments and clinical trials in the Republic of Kazakhstan" approved by Order No. 442 of the Minister of Health of the Republic of Kazakhstan dated July 25, 2007.

Study Design

The animals were divided into four groups of equal numbers, formed by random sampling:

Group 1 – intact (hereinafter – control – Gc);

Group 2 – priming with lead oxide (IV) (hereinafter referred to as "G_{Pb}");

Group 3 – exposure to external gamma radiation (hereinafter referred to as "G_{γ}");

Group 4 – combined exposure to gamma radiation and lead oxide ("$G_{\gamma+Pb}$").

From the moment of its isolation, the control group was in conditions completely similar to the experimental ones, including moving to the radiation site on a radiotherapy device and returning to the vivarium.

In the Pb group, lead (IV) oxide was administered orally for 14 days at 15 mg per1 kg of body weight.

Irradiation of animals was carried out in the radiology department of the Center for Nuclear Medicine and Oncology of Semey, East Kazakhstan region. Animals of the "γ" group were exposed to Co^{60} radiation at the "Teragam" radiotherapy unit. Topometric-dosimetric preparation consists in preliminary preparation of experimental animals for irradiation on the X-ray simulator "Tagasix" in a special cage (a constructed chamber made of organic glass with isolated cells for individual animals), followed by irradiation of a given dose directly on the gamma

therapeutic apparatus “Teragam” manufactured in the Russian Federation (RIP-94.5, field 40x40 cm, power doses of 30.3 r /min, single irradiation at a dose of 6 gy: 700 x-rays, t = 1386 sec.). The size of the field (cell size) corresponds to the maximum radiation field of the radiation therapy unit "Tagasix", i.e. 40×40cm (single irradiation at a dose of 6 Gy: SSD - 97.2 cm, SAD – 100.0 cm, area 40x40 cm, t = 218 sec.). The geometric characteristics of the radiation fields of the simulator “Tagasix” fully correspond characteristics of the gamma-therapeutic device for remote radiation therapy “Teragam”. Instead of the Co^{60} radiation head, it is equipped with a head with an X-ray source. The object is placed on an isocentric therapeutic table, which by its design and parameters corresponds to the therapeutic table of a gamma irradiator [37].

A slice of the pattern of irradiated animals after being displayed on the display screens is directly entered into the planning system via a computer network connection using a digitizer. The calculation of the isodosis was made using the Plan W 2000 planning system. Thus, we obtain a topometric-dosimetric map with technical parameters and planned radiation doses of experimental animals.

Before irradiation of the animals, a topometric-dosimetric calculation was performed, the radiation dose 0.2 G: SSD – 97.2 cm, SAD – 100.0 cm, area 40x40 cm, t = 11 s. Experimental animals were subjected to general gamma irradiation at a dose of 0.2 Gr given as a single dose. During irradiation, the animals were kept in a specially designed organic glass chamber with isolated cells for each animal [38].

In animals of the “γ+Pb” group, irradiation was carried out according to the above scheme on the day of completion of the seed with lead oxide.

Assessment of the Research Results

The slaughter of experimental animals and studies were carried out on the 3rd day after irradiation. During the experiment, all traumatic manipulations in animals were carried out under ether anesthesia. In the tissues of the thymus and spleen, the state of lipid peroxidation (LP) and antioxidant defence (AD) was evaluated. For this purpose, the concentration of primary (diene conjugates – DC) and secondary products (malonic dialdehyde – MDA) of lipid peroxidation was determined. The state of antioxidant protection was assessed by the activity of catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GPO) in the same tissues.

In all experimental animals, indicators characterizing the cellular link of immunity were determined (the number of leukocytes, total lymphocytes, T-lymphocytes (CD3+), CD4+ cells with predominantly helper, CD8+ cells with predominantly killer and suppressor activity, CD4+/CD8+ ratios, phagocytic cells and indicators of their functional activity) [10]. The reaction of inhibition of leukocyte migration (RTML) was carried out. An analysis of the cytokine content in the blood was carried out.

Laboratory tests

Immunological studies were carried out in the Center of the Research Laboratory of the NJSC "MUS" in the immunological department and the biochemical department in the period 2017-2019. Immunological parameters were studied in all experimental animals, the level of cytokines, nonspecific phagocytic resistance of the organism, the state of LP and AD in the lymphoid organs of immunogenesis with separate and combined action of gamma radiation, lead oxide were determined.

- The count of the number of leukocytes was carried out according to a unified method in the counting chamber. 0.02 ml of blood was injected into 0.4 ml of acetic acid. The counting was carried out in Goryaev's chamber in 100 large squares.

- The number of B and T lymphocytes and their subpopulations were determined by immunofluorescence staining of cells using antibodies conjugated with FITC. Reagents: A vial with unconjugated rat monoclonal antibodies also contains BSA (bovine serum albumin) at a concentration of 4 mg/ml, 0.02 M sodium phosphate, 0.3 M sodium chloride and 0.1% sodium azide. Monoclonal antibodies CD3+, CD4+, CD8+, CD19+ FITC manufactured by CALTAG Laboratories (USA) [39].

The course of determination:

1. Lymphocytes were isolated from heparinized blood by centrifugation on a ficoll-verografin gradient (density 1.077 g/ml). We determine the number of lymphocytes, if necessary, dilute the lymphocytic fraction of PBS to a concentration of 2x106 lymphocytes per ml.

2. In a polypropylene centrifuge tube 12x75 mm about 5 ml of a well-mixed suspension of lymphocytes. The cells are deposited by centrifugation at 200 g for 5 minutes. After removal of the supernatant, 50 µl of PBS and 5 µl of monoclonal unconjugated antibodies (produced by CALTAG Laboratories) are added to the driest possible sediment, stirred and incubated for 20 minutes at 4C in the dark. After 2-fold washing, PBS containing 0.2% BSA and 0.1% sodium azide was centrifuged at 200 g for 5 min. After the last washing, the supernatant was removed, leaving the cell sediment dry, 50 µl of diluted PBS polyclonal antibodies to F(ab')2 mouse immunoglobulin $Ig_{(H+L)}$ conjugated with FITC (manufactured by CALTAG Laboratories; No. M35001) were added, mixed and incubated for 20 min on ice. Bottles with polyclonal antibodies contain 700 micrograms of reagent in 1 ml of buffer. To stain 1x106 cells, 1 mcg of polyclonal antibodies conjugated with FITC should be used. Twice washed PBS cells containing 0.2% BSA and 0.1% sodium azide were resuspended in 100 ml of a mixture of PBS: glycerin (1:1); 25 µl of cell suspension was placed on a cooled slide under a cover glass.

Microscopy was performed using an immersion fluorescence microscope (x90 lens) with dimming. The number of antigen-positive cells was determined as the percentage of fluorescent cells observed in the negative control preparation. As a

negative control, preparations prepared in a similar way were used, except that instead of monoclonal antibodies, the cells were treated with Hanks solution.

• The concentration of cytokines in the blood serum: tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-10 (IL-10), γ-interferon was determined by quantitative enzyme immunoassay using the method on the "Uniplan" device (Russia). After receiving the serum, it was quickly frozen and stored at -70˚C. Before the study, serum samples were thawed and filtered through disposable nitrocellulose filters with 0.4 nm pores.

To study the concentration of cytokines in 50 ml of the sample, a commercial test system was used to determine cytokines in experimental animals (rat IFNγ, rat IL-6, rat IL-2, rat TNFa), manufactured by Bender MedSystems GmbH (Bender MedSystems GmbH, Campus Vienna Biocenter 2, Vienna, Austria, Europe) with the assistance of closed-action association "BioHimMak" (Moscow, www.biochemmack.ru) .

The course of enzyme immunoassay. Solutions, standard calibration and control samples (IFN-γ ELISA curve for the rat), the required number of strips for work were prepared. 100 ml of serum dilution solution and 100 ml of standard and control samples were added to the wells, and 100 ml of the studied samples were added to the rest. Incubated for two hours at a temperature of 37 ° C, at 700 RPM. After the end of incubation, the strips were washed 3 times with a solution. At the same time, at least 300 ml of solution was introduced into each well during one washing. The solution from the wells was carefully removed by tapping strips on filter paper, avoiding damage to the inner surface of the microcells. A conjugate solution was prepared 10 minutes before the end of incubation.

100 µl of conjugate was added to each hole of the strips. The strips were placed in a shaker, incubated for 60 minutes at a temperature of 37 ° C, at 700 RPM. A solution of TMB was prepared 10 minutes before the end of incubation. At the end of incubation, the strips were washed three times and moisture was removed. 100 ml of TMB solution was added to each well. The strips were placed in a place protected from light and left at 18-25 ° C for 30 minutes. The reaction was stopped by adding 100 µl of stop reagent to each well and the optical density (OP) was measured. The results of the analyses were taken into account spectrophotometrically at a wavelength of 450 nm. To determine the concentration of cytokines in the samples under study, a calibration curve (graph) was constructed in the coordinates: abscissa axis – cytokine concentration (pg/ml); ordinate axis – the value of the optical density of the sample. A calibration curve was drawn from the obtained points. To determine the cytokine concentration in the analyzed samples, the value of the OP of the analyzed sample was noted on the ordinate axis. A straight line was drawn to the intersection with the calibration curve, a perpendicular was lowered from the obtained intersection point to the abscissa axis. The intersection point and is the desired value of the cytokine concentration. The control sample is used to verify the accuracy and reliability of the results, if the cytokine concentration value calculated according to the calibration schedule in the control sample falls within the limits indicated on the label, it means that the obtained cytokine concentrations in the samples are considered reliable.

Standard calibration dilutions and Bender MedSystems (FlowCytomix) software were used to calculate the concentration of the studied cytokines. The data are presented in pg/ml.

• The reaction of inhibition of leukocyte migration was determined by the method of Artemova A.G. [40]. At the same time, heparinized blood was poured into two wells of 0.2 ml tablet (control and experiment). 0.05 ml of saline solution was added to the control, and 0.05 ml of mitogen was added to the experimental one. Capillaries 4-5 cm long and 1 mm in diameter were filled. The opposite end of the capillary was carefully sealed over the flame of the burner, placed in a centrifuge tube with the sealed end down and centrifuged for 10 minutes at 1000 g. The capillaries were incubated for 18 hours at 37 ° C, after which the level of leukocyte migration in the capillary was assessed under a microscope using an eyepiece micrometer. The migration index (MI) was calculated by the formula (1):

$$MI = O/K, \quad (1)$$

where O is the data of the experimental, K - control tube.

• Determination of circulating immune complexes (CIC) was carried out by the Grinkevich method [41]. The method is based on selective precipitation of antigen-antibody complexes in a 3.75% solution of polyethylene glycol, followed by photometric determination of the density of the precipitate. To determine the CEC, blood taken without anticoagulants was kept for three hours in a thermostat at 37 ° C. Then, to separate the serum, the blood was centrifuged at room temperature for 30 min (1500 rpm). The optical density of serum samples was determined in a borate buffer and in a Polyethylene Glycol-600 reagent on a SF-26 spectrophotometer, in 10x10 mm cuvettes, at a wavelength of 450 nm. The results were expressed in units of optical density.

• The content of phagocytic polynuclears (neutrophils and pseudo-eosinophils) was determined using latex as a phagocytic material. The phagocytic indicator was considered to be the percentage of neutrophils that entered phagocytosis from the total number of neutrophils [].

Heparinized blood (20-25 units/ml) was poured into centrifuge tubes in a volume of 0.2 ml. 0.1 ml of latex was added to the test tube, 0.1 ml of 0.89% NaCl solution was added to the control tube. The suspension was stirred by shaking, the tubes were closed and kept in a thermostat at 37 ° C for 30 minutes (repeated shaking every 10-15 minutes). After the end of incubation, the tubes were cooled with running water for 7 minutes. The supernatant was removed, the precipitate was resuspended in the liquid residue and smears were prepared (Romanovsky-Giemse staining, immersion microscopy, 10x90 magnification). For 100 calculated neutrophils, the number of phagocytized latex neutrophils was determined, which was expressed as a percentage.

• The determination of the mononuclear phagocytic system parameters (NST test) was carried out using the Nagoev method (spectrophotometric method) [42]. The method allows to determine the total redox activity of phagocytes due to the energy and products of metabolic respiratory explosion.

0.2 ml of heparinized blood was taken into a test tube, after which 0.1 ml of nitrosine tetrazolium reagent (NST) was added. The contents of the test tube, kept for 1.5 hours in a thermostat at a temperature of 37C, were centrifuged at 1500 rpm for 5 minutes. After removing the filler fluid, a smear of sediment was made, which was stained with safronin and fixed with a mixture of ether and alcohol until completely dry. After holding the smear for 20 minutes, it was washed with running water. Cells with colored granules were counted, the HCT test was calculated as a percentage of all phagocytes.

Biochemical tests

After decapitation of animals, their organs were crushed, placed in a cooled solution (0°C) 0.25 M sucrose. After cooling, the tissues were thoroughly washed in a cooled 0.25 M sucrose solution until traces of blood were removed. Then they were homogenized in the cold in a Potter glass homogenizer with a Teflon pestle in 0.25 M sucrose (8 ml per 1 g of tissue). During homogenization of liver tissue, the rotation of the pistil is 600 rpm for 25 seconds. Tissue homogenates were filtered through a layer of sterile gauze and centrifuged at 3000 rpm for 30 minutes (t=0-2°C). Test tubes with homogenates were kept in ice throughout the study of enzyme activity. In the experiment, a filler fluid was used.

Determination of primary products of lipid peroxidation: Diene conjugates (DC) (Gavrilov, Mishkorudnaya) [43].

DC was previously extracted from blood plasma using isopropylheptan (Z.Placer et al., 1982). To 0.2 ml of blood serum, add 4.0 ml of isopropyl heptane, stir on a magnetic stirrer for 5 minutes, add 1.0 ml of 0.1M HCl solution, shake for 5 seconds, then add 2.0 ml of heptane, shake for 15 seconds, then centrifuged for 10 minutes (1500 g). 0.5 ml of distilled H2O was added to the control sample instead of blood serum. The calculation was performed taking into account the molar extinction coefficient equal to 2.2*104 M-1 cm-1 relative units of optical density D 233 nm per 1 ml of plasma. Normal DC values in the plasma of a healthy person are 2.2–2.6 RU/ml

Determination of secondary products of lipid peroxidation: malondialdehyde [44].

0.8 ml of H2O (dis) and 1.0 ml of 0.6% thiobarbituric acid solution were added to 0.2 ml of tissue homogenate. The samples were incubated in a boiling water bath for 30 minutes and cooled at room temperature. 1.0 ml of 5% KON and 2.0 ml of isopropyl alcohol were added to extract the colored alcohol, the reaction product, and centrifuged for 20 min at 8000 rpm. In the supernatant, the optical density was determined at λ = 520 nm, on SF-26, against the control, a cuvette of 1 cm. For control, H2O (dis) was added to the sample instead of tissue homogenate.

The determination of glutathione reductase (GR) [45] was determined by the level of

NADP.H2 (spectometrically) after 10 min (3).

NADF.H2 + glu-S-S-glu – 2HS-glu+ NADP

(3)

The prepared mixture in test tubes was placed in a thermostat (37C) for 15 minutes and transferred to the experimental and control cuvette of the spectrophotometer, where 0.2 ml of hemolysate was added (1:40) and mixed thoroughly. Spectrophotometry was performed for 10 min with an interval of 1 min (λ=340 nm). The control was distinguished by the absence of NADPH in the sample. The unit of measurement is mmol NADP*H2/ml.eritots/min. Determination of glutathione peroxidase GPO (477). The activity of GPO is determined by the accumulation of oxidized glutathione. The wavelength of SF is 260 nm.

The incubation mixture was incubated in a thermostat (t= 37C, 15 min). 0.5 ml 1.8 mmol of H2O2 solution was alternately added to the experimental and control samples. After 2 minutes, the reaction was stopped by adding 1 ml of 10% TCU. They were centrifuged for 15 minutes at 3000 rpm, after which the optical density on the SF of the filler fluid (λ = 260 nm) was determined. The control contained all components except H2O2. SF activity of GPO was expressed in mmol of oxidized glutathione/ml.

The determination of catalase activity [46] is based on the ability of hydrogen peroxide to form a stable colored complex with molybdenum salts.

Catalase is widespread in humans and animals, with the largest amounts of the enzyme found in red blood cells, liver and kidneys. Catalase activity is also determined in all plants and microorganisms, with the exception of obligate anaerobes. The function of the enzyme is to prevent the accumulation of hydrogen peroxide formed during the dismutation of the superoxide anion and during the aerobic oxidation of reduced flavoproteins. Catalase belongs to the enzymes that retain their high activity for the longest time, almost does not require activation energy, the reaction rate of this enzyme limits only the rate of diffusion of the substrate to the active center.

Statistical analysis

When analyzing the data obtained, a nonparametric method was used – the Mann-Whitney difference significance criterion. The evaluation of the relationship of qualitative characteristics by the magnitude of inertia and the evaluation of the significance of this relationship was carried out by calculating the Spearman correlation coefficient. $P<0.05$ was taken as the boundary criterion of statistical significance for refuting the null hypothesis.

Results

We evaluated the indicators of the cellular link of immunity depending on the type of exposure to the body (Table 1).

Table 1 – Indicators of cellular immunity in the studied groups of experimental animals

Indicator	Group											
	Gc			G_{γ}			G_{Pb}			$G_{\gamma+Pb}$		
	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75
Leucocytes, $*10^9$/мл	6.9	7.3	7.8	7.3	8.0	8.5	7.2	8.0	8.6	6.5	7.1	7.8
Lymphocytes, $*10^9$/ml	2.9	3.3	3.6	2.8	3.1	3.5	3.0	3.4	3.9	2.7	3.0	3.3
%	44.7			38.8			42.1			42.3		
CD3+, $*10^9$/ml	2.1	2.5	2.9	1.7	2.0	2.4	1.9	2.2	2.6	1.5	1.9*	2.2
%	34.1			25.3			27.7			26.6		
CD4+, $*10^9$/ml	1.4	1.6	1.8	1.0	1.2*	1.3	1.1	1.3*	1.4	0.8	1.1*	0.2
%	21.7			15.2			16.0			15.5		
CD8+, $*10^9$/ml	0.6	0.8	0.9	0.5	0.6*	0.7	0.6	0.7	0.8	0.4	0.6*	0.7
%	11.0			7.5			8.7			8.5		
CD4+/CD8+	2.02			1.98			1.86			1.83		
LMS, RU	18.3	21.5	25.0	14.9	17.4*	20.4	20.2	22.6	25.0	13.1	14.3*	16.4
Note: * - differences with the control group are significant, a detailed description in the text												

The content of leukocytes in the blood of animals of the selected groups did not significantly depend on the impact on them. There was a tendency to exceed this indicator when exposed to radiation and lead compounds, but not when combined.

There were also no differences in the number of lymphocytes, nor was there a single trend in relation to this indicator. The absolute and relative content of T-lymphocytes decreased under the influence of both negative factors, and significantly – only with a combination of those (by 23.7%, p=0.03).

These features at the time of completion of the experiment were determined in relation to the lymphocytes of both studied differentiation clusters. CD4+ content indicators were lower than in the control group by 24.7% under irradiation, by 18.9% - with lead oxide priming and by 31.4% - with their combination (p=0.021, p=0.043 and p=0.015, respectively). A significant decrease in the number of CD8+ was determined in both groups of exposure to ionizing radiation, while in the group of combined influence of negative factors there were no differences from isolated gamma irradiation (differences with control by 24.3% and 25.7%, p=0.035; p=0.033).

The index of inhibition of leukocyte migration during irradiation significantly decreased. In addition, the additional introduction of lead into the body caused a greater severity of changes. The differences with the control were 19.2% and 33.6%, respectively (p=0.045; p=0.028).

Table 2 presents data on the functional state of the phagocytic link.

Table 2 Indicators of phagocytosis in the studied groups of experimental animals

Indicator	Group											
	Gc			G_{γ}			G_{Pb}			$G_{\gamma+Pb}$		
	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75
Phagocytosis, %	36	44	51	39	47	54	40	47	55	35	43	51
Phagocytic number, RU	3.1	3.7	4.3	2.5	2.7*	3.0	3.0	3.8	4.4	2.2	2.5*	2.8
NST-spontaneous test, RU	5.9	6.5	7.1	4.7	5.2	5.8	5.2	5.8	6.3	3.0	3.4*	3.7
Note: * - differences with the control group are significant, a detailed description in the text												

The number of phagocytic cells had no significant differences between the compared groups.

The indicator of their functional activity – the phagocytic number – had a decrease under the action of ionizing radiation. The median value of the indicator in the group of irradiated animals was 72.6% of the control level, and in combination with Pb seed – 67.8% (p=0.043, p=0.037).

The indicator of the production of reactive oxygen species by phagocytic cells was significantly lower than in the control, only in one group – combined exposure. At the same time, the differences reached 47.8% (p=0.007).

The content of cytokines in the blood of animals depends on many factors, including the functional status of immune cells (both cytokine-producing and regulatory. It is quite difficult to assess the essence of the changes associated with the regulation of the production of these compounds, but their content in the body and the relationship between various cytokines determine the possibility of the development of pathological processes and even diseases [12].

Table 3 presents data characterizing the content of some cytokines in the blood of animals of experimental groups.

Table 3. Indicators of cytokine content in the blood of experimental animals

Indicator	Group											
	Gc			G_{γ}			G_{Pb}			$G_{\gamma+Pb}$		
	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75
IL-1, pg/ml	2.2	2.6	2.9	2.1	2.2	2.4	1.8	2.1*	2.3	1.7	1.9*	2.0
IL-2, pg/ml	3.0	3.5	4.1	3.1	3.7	4.3	2.9	3.3	3.8	2.0	2.6*	3.2
IL-6, pg/ml	4.1	4.7	5.4	3.7	4.1	4.7	5.0	5.8	6.5	3.9	4.4	5.0
IL-10, pg/ml	9.7	12.2	14.2	7.3	9.9	11.8	5.9	7.6*	9.4	4.7	5.9*	7.0
TNFα, pg/ml	5.9	7.1	9.3	5.2	6.0	6.9	8.8	10.4*	12.1	4.6	5.6*	6.4
Note: * - differences with the control group are significant, a detailed description in the text												

In terms of IL-1 content, a significant decrease was determined when experimental animals were primed with lead oxide and with combined exposure. The differences with the control were 19.4% in the first case and 26.1% in the second (p=0.040 and p=0.019, respectively).

The concentration of IL-2 showed a decrease in $G_{\gamma+Pb}$. the differences with the control reached 25.9% (p=0.037). There were no significant differences in the analysis of the IL-6 content in the blood. IL-10 level showed a significant decrease in the groups of exposure to lead compounds and combination with irradiation. When exposed to lead, there was an increase in the TNFa content relative to Gc, but the opposite change for $G_{\gamma+Pb}$ was observed (a decrease in the indicator relative to the control, p=0.027).

Based on the data of early studies by other authors and our own results, we formulated a hypothesis about the relationship of the state of oxidative and antioxidant mechanisms in the tissues of the organs of immunogenesis with the state of immunocytes. To test it, a study of these parameters was undertaken in the presence of toxic, radiation exposure and their combination and a comparison of the results obtained with the reactions of various parts of the immune system (Table 4).

Table 4 – Features of LP and AD indicators in thymus tissues in experimental animals

Indicator	Group											
	Gc			G_{γ}			G_{Pb}			$G_{\gamma+Pb}$		
	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75
DC, RU/ml	1.39	1.71	1.97	1.56	1.99*	2.35	1.98	2.46*	2.89	4.12	4.80*	5.40
MDA, nM per 1 mg OL	0.03	0.04	0.05	0.04	0.06	0.07	0.04	0.05	0.06	0.07	0.09*	0.11
GPO, nM/min. per 1 mg of protein	1.08	1.40	1.77	1.32	1.76	2.09	1.13	1.59	2.03	0.75	1.22	1.64
GR, nM/min. per 1 mg of protein	0.30	0.35	0.39	0.23	0.28	0.32	0.30	0.37	0.41	0.15	0.21*	0.25
CAT, EA	20.2	26.4	30.7	21.5	28.5	34.9	27.6	31.8	37.0	19.9	25.0	30.7
Note: * - differences with the control group are significant, a detailed description in the text												

The content of primary and secondary lipoperoxidation products when determining them in the selected groups of experimental animals had significant differences with the control. Thus, the median concentration of DC exceeded the corresponding indicator of the control group by 16.5% during irradiation, by 44.0% - when lead oxide was injected into the body and by 180.9% - with combined exposure (p=0.040; p=0.019 and p<0.001), respectively. It is noticeable that the combined effect caused a sharp excess of the production of primary metabolites of the oxidative process (DC), which have a high reactivity and toxic effect. The effect of antioxidant mechanisms was probably the cause of a less pronounced increase in the content of MDA in animals subjected to experimental exposure. A significant excess over the control indicator was determined only in

the group of a combination of oral administration of lead and low-dose irradiation (p=0.029).

Analysis of the state of antioxidant mechanisms allowed us to conclude that the factors used in the experiment had a significantly lower effect on their activity in thymus tissues. Thus, we identified only one parameter for which significant differences with the control were recorded – the activity of GR in the group of combined exposure, which was 39.7% lower (p=0.025).

Similar indicators of LP and AD were analyzed in fresh samples of spleen tissues (Table 5).

Table 5. Features of LP and AD indicators in spleen tissues in experimental animals

Indicator	Group											
	Gc			G_{γ}			G_{Pb}			$G_{\gamma+Pb}$		
	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75	Q25	Me	Q75
DC, RU/ml	2.08	2.77	3.41	2.46	3.15	3.73	3.08	3.99*	4.82	4.55	5.38*	6.20
MDA, nM per 1 mg OL	0.06	0.08	0.11	0.05	0.07	0.09	0.10	0.14*	0.19	0.17	0.25*	0.34
GPO, nM/min. per 1 mg of protein	1.85	2.12	2.47	3.01	3.46*	3.88	2.59	3.17*	3.72	1.88	2.43	2.81
GR, nM/min. per 1 mg of protein	0.59	0.66	0.73	0.88	1.02	1.17	0.79	0.93	1.20	0.55	0.64*	0.74
CAT, EA	23.4	28.9	35.3	30.7	35.1	39.2	22.8	27.5	32.4	19.9	25.3	31.3
Note: * - differences with the control group are significant, a detailed description in the text												

Conclusions

To achieve this goal, it is necessary to study in an experiment the separate and combined effect of gamma radiation at a dose of 0.2 gr and lead oxide on the immunological reactivity of the body, the state of nonspecific phagocytic resistance, LP and AD in primary and secondary lymphoid organs of immunogenesis and cytokine profile. In three series of experiments on white mongrel sexually mature rats, the reaction of the T-system of immunity under the separate and combined action of stress factors was studied.

With simultaneous effects of damaging factors on the body, it is theoretically possible to sum up or potentiate unidirectional lesions. At the same time, the weighting factor increases the damage threshold of many systems, which was not reached with the isolated action of each of these components. Of great interest are the reaction of the altered immunological reactivity of the body to the action of lead oxide, the combined effect of a small dose of gamma radiation and lead oxide, and the development of a general adaptation syndrome [30].

Experimental studies in two series on white mongrel sexually mature rats allowed us to reveal that in the immune system under the influence of gamma radiation there are dysregulatory disorders of immunity with an increase in T-suppressors

and a decrease in T-helpers, inhibition of cellular response to stimulation of PHA. When lead oxide was seeded in the cellular immunity link against the background of leukocytosis, the absolute content of CD4+ and CD8+ lymphocytes increased, the relative number of CD8+ lymphocytes subpopulation did not undergo significant changes, the relative numbers of CD4 lymphocytes subpopulation decreased, the immunoregulatory index and the leukocyte migration index in the LMS reaction to PHA migration decreased.

The resulting manifestations are apparently related to the pathogenetic features of the effects of radiation and the damaging effect of lead oxide on T-lymphocytes, causing their death, impaired differentiation and expression of surface receptors, blockade of receptors by physiologically active substances, pathological metabolites, immune complexes. Another possible mechanism for the development of T-lymphopenia may be the redistribution of lymphoid cells between blood and organs, that is, a change in the functional activity of the lymphoid system and the migration of its cells from the thymus and spleen to the bone marrow and back [31], which is apparently due to the damaging effect of radiation factor and lead oxide on radiosensitive lymphoid tissues.

Leukocytosis indicates the migration of lymphoid cells and stress redistribution of blood against the background of hematopoietic tissues. On the other hand, an increase in the number of lymphocytes in peripheral blood may be associated with the maximum involvement of these cells in the immune process when exposed to the toxic effects of lead oxide and the appearance in peripheral blood of a significant proportion of immature lymphoid cells that are not capable of rosette formation and full-fledged functions due to the blockade of receptors by physiological active substances or pathological metabolites. In addition, the T-lymphocytes themselves can have a direct damaging effect on tissues (natural killers in the early stages of GAS) and, as delayed hypersensitivity develops, they can be one of the components of pathogenetic factors of organ lesions and autoallergic processes during irradiation and toxic lead oxide damage. At the same time, it cannot be excluded that the pathogenic effect of T-lymphocytes also has a protective value, contributing to a certain extent to the elimination of damaged tissue elements. It was found that the low level of lymphokine-producing ability of leukocytes, determined by the decrease in the migration index in LMS for PHA migration, reflects the low functional activity of the T-immune system. In our studies, in both series, in addition to a decrease in the quantitative indicator, the functional activity of the T-system of immunity also changes. A decrease in the lymphokine-producing ability of leukocytes is characterized by a decrease in the migration index in LMS on PHA migration from 21.5±0.01 to 18.0±0.4 with gamma irradiation and to 19.6±0.3 ($P<0.05$) with lead oxide seed.

With the combined effect of lead oxide on an organism irradiated with a small dose of gamma radiation, the total number of leukocytes and lymphocytes in peripheral blood normalizes in the T-immune system, the absolute and relative number of CD8+ lymphocytes subpopulation increases, and the lymphokine-producing ability of leukocytes increases. The results of experiments show that when lead oxide acts on an organism irradiated with a small dose of gamma radiation, the number of T cells with helper activity increases, which increases the proliferative activity of T cells, as a result of which their functional activity

increases. With the separate action of gamma radiation and lead oxide, there is an increase in T cells with suppressor activity, which causes a decrease in the functional activity of the cellular link of immunity. Hence, their direct effect on the cellular link of immunity follows, and with the combined action of stress factors, there is no potentiation, but on the contrary, an antagonism of interrelated specific and nonspecific manifestations. To confirm this phenomenon in the T-system of immunity, we investigated the state of LP and AD in the thymus under the influence of combined and separate action of gamma radiation and lead oxide [32].

The results of the experiments showed that under the influence of a small dose of gamma radiation in the thymus, the activation of LP and the voltage of AD occurs, the latter manifests itself in a decrease in the production of GR and an increase in the content of catalase

In the thymus, lead oxide reduces the production of GP, GPO and catalase and increases the activity of LP. The combined effect of lead oxide and gamma radiation in the thymus does not cause the activation of LP.

It is known that the pathogenesis of stress dysfunction is caused by an increase in the generation of oxygen radicals and, as a consequence, a violation of the normal ratio of pro- and antioxidants in tissues. These processes appear to be the most significant in the tissues of lymphoid organs of immunogenesis, which is associated with an excess of free oxygen and a deficiency of antioxidant enzymes in lymphoid organs [13,15,17].

The LP process itself develops in the form of chain reactions in the lipid phase of membranes and lipoproteins, the initial stages of this complex system of reactions occur in the aqueous phase [14,16].

One of the primary reactions when exposed to ionizing radiation is the development of free radical LP processes, the end products of which are toxic substances. According to a number of researchers, the resulting radiotoxins, acting on the components of the cell membrane, numerous enzymes and the genetic apparatus of the cell, contribute to the development of radiation damage, and the radiosensitivity of the organism of individual organs is largely determined by the initial level of active products of free radical oxidation in the organs, depending on the timing of the study and the dose [18,21], since when considering the pathogenetic mechanisms of the adaptation syndrome, we traced the dynamics of the content of primary and secondary products of lipid peroxidation and antioxidant defense enzymes.

The possibility of the development of various pathological processes under the action of LP processes is associated with the biological activity of compounds that are formed during the reactions and with the role of biomembranes, the structural component of which are lipids. [19,20]. LP is one of the factors leading to impaired physiological functions under stress [22]. In rats, an increase in the processes of free radical oxidation under stress is associated with an increase in the reactivity of the neuro-endocrine system and with a high level of oxygen and fatty acids in the tissues, as well as with a decrease in the functional activity of

the antioxidant system. The stress load leads to the activation of competitive free radical oxidation of acids and triglycerides that have not been used for a long time in the course of enzymatic oxidation [23].

In our experiments, in the thymus, with the separate action of a small dose of gamma radiation and lead oxide, the activation of LP occurs, in the first case, the stress of antioxidant enzymes occurs in the AD system, in the second case, a decrease in their activity causes an increase in the contents of primary and secondary products of lipid peroxidation.

Despite the decrease in the production of Gr and GPO in the thymus with the combined effect of radiation and lead oxide, the formation of excess lipoperoxidation products is not observed, apparently, the balance of the oxidant and antioxidant systems is not disturbed due to the high compensatory concentration and activity of catalase, it is also possible that the combined effect of stress factors reduces the reactivity of the neuro-endocrine system with low the level of oxygen and fatty acids in the tissues. The data obtained indicate the absence of metabolic and morphological disorders in the membranes of thymus cells. This, apparently, supports the high functional activity of the T-immune system, with the combined effect of lead oxide on the body irradiated with gamma rays in a small dose.

Data on the study of the humoral link of immunity in the conditions of exposure to ionizing radiation and lead oxide on the body is the subject of discussion. Taking into account the complexity of the nature of changes in the humoral link of immunity under various types of stress factors, we set the task in an experiment to study the reaction of secondary lymphoid organs of immunogenesis – the spleen and liver in connection with the nonspecific phagocytic link of immunity, cytokine profile, directly involved in the regulation and coordination of the humoral link of immunity to assess the functional state of the humoral link of immunity, we studied the state of LP and AD in secondary lymphoid organs of immunogenesis – in the spleen and liver, responsible for the coordination and regulation of the humoral link of immunity.

An integral part of oxidative metabolism is LP, which is one of the pathogenesis factors of a number of diseases and is part of the adaptive mechanism of the body [23,26,29]. As a result of the activation of LP and the accumulation of free radicals, the structural and functional integrity of cell membranes is disrupted, lysosomal enzymes are released, which eventually leads to pathological processes in the cell and in the body as a whole [24]. The substrate for LP is polyunsaturated fatty acids. Diene conjugates, which are the primary products of peroxidation, increase the polarity of hydrophobic hydrocarbon tails of fatty acids forming the lipid layer of biological membranes. During the physiological course of the process, the areas of hydrocarbon membranes whose polarity has increased are displaced from the deep layers of the membrane to its surface, thereby facilitating the process of self-renewal of the membrane and influencing its permeability and ion transport [25]. With an excess of free radical oxygen forms, self-accelerating LP leads to the complete destruction of unsaturated fatty acids, disruption of the structure and function of proteins, and as a consequence, leads to cell death [28]. Any adaptive or pathological process proceeds against the

background of the formation of reactive oxygen species (ROS) and the intensification of free radical oxidation of biosubstrates. Lipid oxidation, activation of LP is one of the factors of violation of physiological functions under stress.

The results of numerous observations have shown that oxidative stress plays an important role in the pathogenesis of many disorders, which develops due to an imbalance between the oxidant and antioxidant systems. At the same time, lipid peroxidation products – diene conjugates and malondialdehyde, which are destabilizers of cell membranes, reach high concentrations in the blood and tissues.

The glutathione link of the antioxidant system is the main system of protection against lipid peroxidation, eliminating lipid peroxides and hydrogen peroxide, but it is known that the low level of activity of catalase, neutralizing hydrogen peroxide, against the background of a high content of malondialdehyde enhances the toxic effect of lipid peroxidation, as well as the activation of lipoperoxidation in tissues is associated with microcirculation disorder, the development of hypoxia and impaired functioning of the mitochondrial respiratory chain under stress. Mitochondria play a key role in energy metabolism, a source of reactive oxygen species, regulates the activity of ion channels, and the initiation of cytoprotective mechanisms under stress. Mitochondrial dysfunction leads to pathologies and even death, and mitochondrial DNA is less protected from stressful influences than nuclear DNA, which stress induces oxidative DNA modifications, chromosomal aberrations and death of body cells.

The main metabolite of the superoxide radical dismutase reaction is hydrogen peroxide (H2O2), which, as a strong oxidizer, can have a toxic effect on cells. Maintaining the normal level of hydrogen peroxide is provided by enzymes that catalyze the destruction of hydrogen peroxide molecules is catalase and a group of hydrogen enzymes. These are peroxidases, including glutathione peroxidase (GPO). Maintenance of a sufficient level of reduced glutathione, oxidized during the functioning of glutathione-dependent antiperoxide systems, is provided by glutathione reductase (GR). As a result, an increase in the primary and secondary products of lipid peroxidation was found, with the intensity of the activity of antioxidant enzymes-glutathione peroxidase (GPO) and catalase activity (CAT). Catalase reduces the level of ROS through the inactivation of hydrogen peroxide. Inactivation of hydrogen peroxide leads to an increase in the activity of mitochondrial RNA catalase in vitro. Thus, an increase in catalase activity is a very important point for preventing the formation of reactive oxygen species [33].

Therefore, it can be assumed that with the combined action of stress factors, the depletion of antioxidant resources in the antioxidant system occurs, both in the central and peripheral lymphoid organs of immunogenesis.

The existence of an initial rise in the intensity of LP is explained by the radiation induction of the mass of active oxidative products, its decrease can be explained by the accidental release into the circulation of products with antioxidant and antiradical activity (catecholamines and glucocorticoids).

The actual experimental material showed that with the separate effects of a small dose of gamma radiation and the combined effect of radiation and lead oxide in the liver, there was no activation of LP, the change in AD was manifested in a decrease in the catalase content. This indicates that with the separate and combined action of gamma radiation in a small dose and lead oxide in the liver, there is no imbalance between the oxidant / prooxidant systems. Under the influence of lead oxide in the liver, there is a decrease in AD and activation of LP. The decrease in the production of GR and GPO led to an imbalance of the oxidant-prooxidant system with the subsequent development of oxidative stress. The development of oxidative stress in the spleen and liver was observed with the separate action of lead oxide, with the combined effect of lead oxide on the body irradiated with gamma rays in a small dose, the development of oxidative stress was observed in the spleen, there was no increase in the content of primary and secondary lipoperoxidation products in the liver. According to the state of LP and AD, it can be stated that the liver is more resistant to the separate and combined action of lead oxide in comparison with the spleen [34].

The existence of an initial rise in the intensity of LP is explained by the radiation induction of the mass of active oxidative products, while its sharp decrease can be explained by the accidental release into the circulation of products with antioxidant and antiradical activity (catecholamines and glucocorticoids). Thus, it can be assumed that the initial rise in the intensity of LP, due to the appearance of a mass of reactive oxygen species, induces an organism's response in the form of the release into circulation of humoral products of homeostasis regulation systems with antioxidant activity.

In our experiments, serious changes were observed on the part of the nonspecific phagocytic link of immunity, with the separate and combined action of lead oxide and gamma radiation at a dose of 0.2 Gy. Under the action of a low dose of gamma radiation and its combination with lead oxide, a decrease in the nonspecific phagocytic activity of the body is due to a decrease in the phagocytic activity of neutrophils and leukocytes.

With the separate action of lead oxide, a decrease in the nonspecific phagocytic resistance of the organism; mainly due to a decrease in the morphofunctional phagocytic activity of neutrophils, the data obtained indicates a decrease in the nonspecific phagocytic resistance of the organism both with separate and combined action of radiation and lead oxide. It is possible that a decrease in the phagocytic activity of leukocytes may, along with a protective effect, have the value of feedback mechanisms. It is believed [33] that activated phagocytes cause significant suppression of T- and B-lymphocytic mechanisms of immunity. It is obvious that the damaging effect of CIC occurs with a decrease in the activity of phagocytic cells, however, this effect is realized directly mainly through the activation of complement. Under the influence of lead oxide on an organism irradiated with gamma radiation at a dose of 0.2 Gy, it has a suppressive effect on the humoral link of the immune system, which, of course, causes the development of a secondary immunodeficiency state of radiation and post-stress genesis. The results of the analysis show that with the separate action of a small dose of gamma radiation, the content of IL-1 increases by 4.2 times, IL-10 by 6.0 times in comparison with the initial data, the production of IL-2, IL-6, IFN-γ is

reduced by 2.9, 2.13 and 4.73 times, respectively. With the separate action of lead oxide, the production of IL-1, IL-10 was significantly increased by 2.0, 2.25 times, the production of IL-2, IL-6 was reduced by 1.75 and 1.56 times, respectively, no significant changes were noted on the part of TNF and IFN-γ. The following changes occurred in the cytokine profile under the combined effect of gamma radiation and lead oxide: an increase in the production of IL-1 and IL-10, TNF by 2.84, 2.5 and 1.81 times, respectively. Reduction of IL-2 production by 1.75 times, IL-6 by 2.35 and IFN-γ by 2.6 times.

An increase in the functional capacity of T-lymphocytes and lymphocytosis indicates in favor of stimulating an immune response and/or an active response to radiation. There is evidence in the literature that a stimulating effect on the proliferative activity of lymphocytes is found in populations of splenocytes and thymocytes /24-28/. As a result of the action of a small dose, quantitative and qualitative changes occur in the T-system of immunity, which took place in our experiments.

An active response to radiation means the induction of a reaction caused by the radiation damage itself or the consequences of these injuries. Although an active response to radiation occurs after exposure to high doses of radiation, the active response in our experiments can be attributed to the combined effect of radiation and lead oxide, causing an intensification of the lymphokine-synthesizing ability of leukocytes in the reaction of LMS to PHA migration. Thus, after gamma radiation at a dose of 0.2 Gy and the action of lead oxide, the concentration of CIC in the blood serum increases, the relative and absolute number of CD19+ lymphocytes subpopulation decreases. According to the results obtained, it can be judged that the combined effect of radiation and lead oxide causes the activation of LP in the spleen, with a decrease in nonspecific phagocytic resistance of the body causes depression of the humoral link of immunity [26,35].

Summarizing the data obtained on the effect of separate and combined effects of radiation and lead oxide, the following conclusion should be made: the combined effect of stress factors causes quantitative and qualitative changes in the T-system of immunity; suppression of the humoral link of immunity; reduction of nonspecific phagocytic resistance of the body. The revealed disorders in the state of the immune system with various separate and combined effects of radiation and lead oxide raises the question of indications for their correction with the search for effective means of its implementation.

Discussion

Immunological aspects of adaptation under the conditions of a low dose of gamma radiation in the cellular immunity link are characterized by dysregulatory disorders of immunity with an increase in T-suppressors and a decrease in T-helpers, inhibition of the cellular response to PHA stimulation, tension of the humoral immunity link, activation of LP in the thymus, increased IL-1 and IL-10 content and a decrease in nonspecific phagocytic resistance of the body.

Under the action of lead oxide in the cellular and humoral links of immunity, quantitative and qualitative indicators increase and decrease, in the thymus, liver

and spleen, the activation of LP is due to a decrease in antioxidant protection, the production of IL-1, IL-10 is increased, the production of IL-2, IL-6 and nonspecific phagocytic resistance of the body are reduced. The effect of lead oxide on an organism irradiated with gamma radiation at a dose of 0.2 Gy is manifested by suppression of the humoral link of the immune system, which, of course, causes the development of a secondary immunodeficiency state of radiation and post-stress genesis.

With the combined action of gamma radiation and lead oxide, there is an increase in T-cells with helper activity and qualitative indicators of the T-system of immunity. In the cytokine profile, there is a predominance of production towards pro-inflammatory cytokines, the phagocytic activity of leukocytes and neutrophils decreases

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