

The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS), 2025

Volume 17, Pages 27-34

ICGeHeS 2025: International Conference on General Health Sciences

Acute ^{60}Co Γ -Irradiation Effect on Lipid Peroxidation and Immune System in Experimental Animals

Oralbek Ilderbayev

L.N. Gumilyov Eurasian National University

Kerim Mutig

I.M. Sechenov First Moscow State Medical University

Gulzhan Ilderbayeva

L.N. Gumilyov Eurasian National University

Darkhan Uzbekov

L.N. Gumilyov Eurasian National University

Aruzhan Ilderbayeva

University of Duisburg-Essen

Ainur Zhanilova

L.N. Gumilyov Eurasian National University

Abstract: The objective of the study was to explore the biochemical and immunological indicators in experimentally irradiated animals. Group I - intact, group II - exposed to γ -radiation with a dose 6 Gy. The parameters characterizing the body's immune system were studied, lipid peroxidation outputs and antioxidant protective enzymes activity in blood lymphocytes were identified. The results of the study showed that exposure to a sublethal dose of radiation entails a reduction in cellular immunity, especially T-lymphocytes and their subpopulation, as well as to a decrease in the functional activity of the body's non-specific defense. Leukopenia and lymphopenia were observed in irradiated animals. The same pattern was revealed for T-lymphocytes: the number decreased by 20.25%, leading to a fall in CD4⁺ cells. It was also found that T-suppressors number declined by 14.28%. Ionizing radiation effect led to an increase in the level of DC and MDA, inhibition of the activity of glutathione reductase, glutathione peroxidase and catalase enzymes, as a result of which the oxidative stress formation in the body was observed. Suppression of cellular and humoral immunity, non-specific protection of the body and imbalance of LPO-AOP create preconditions for the emergence of immune pathological state of radiation origin, indicating the necessity to develop promising for adaptation correcting methods.

Keywords: Radiation, Lipid peroxidation, Antioxidant system, Immunity

Introduction

The immune system state plays a pivotal role in determining the body's ability to resist adverse environmental influences. Immune mechanisms are closely linked to other human physiological systems, and their dysfunction inevitably has a detrimental effect on the general condition of the body. Additionally, the immune system constitutes one of the main targets for various pathogenic factors (Pennington et al., 2005).

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- Selection and peer-review under responsibility of the Organizing Committee of the Conference

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The biological effectiveness of ionizing radiation significantly exceeds the performance of all known modes of radiation (Kuperman, 2018). There is not a single vital function of the body that would not be suppressed by radiation. However, the sensitivity of organs and tissues to ionizing radiation varies, as does their role in radiation pathology and the final consequences of radiation sickness. The most susceptible to radiation are proliferating cells involved in hematopoiesis, while radiation-physiological alterations that disrupt the functioning of hematopoietic organs and blood cells lead to changes in the human immune system. The later of those who live and work in conditions of long-term exposure to radiation of various origins suffers especially (Burlakova, 2004).

Ionizing radiation affect biological systems entails changes in almost all their entities. Immunological reactivity is defined as one of the most radiosensitive functions in both humans and animals. One of the characteristic features of radiation exposure is the long-term preservation of damage in individual components of the immune system, inducing long-term consequences and complications, including the development of malignant neoplasms.

Ionizing radiation can affect not only the differentiation of T-helper cells, but also other components of the immune response. For instance, exposure to ionizing radiation can activate macrophages and dendritic cells, which in turn changes the profile of cytokines released in the tumor microenvironment. This can provoke the antitumor immune response suppression and contribute to disease progression. In addition, an imbalance between T-helper subtypes can hinder the formation of an effective immune response memory, which is a critical aspect for achieving long-term outcomes after radiotherapy. Studies show that modification of the immune response towards a more active Th1 response can improve treatment outcomes. Thus, understanding the mechanisms underlying the immune response deteriorations under the influence of ionizing radiation discloses contemporary perspectives for the development of combination therapies that can improve the effectiveness of radiotherapy through immunotherapy or other approaches aimed at restoring the balance between T-helper subtypes. Exposure to ionizing radiation often induces T-helper cell differentiation, resulting in an imbalance of Th1 and Th2 cell subtypes that may cast a shadow over the effectiveness of cancer radiotherapy (Gao et al., 2018).

The biological effects of radiation comprised by atomic and molecular ionization and excitation in organs and tissues, leading to the formation of highly active radicals and peroxides [Shen et al., 2018]. The initial three phases of ionization occur at the molecular level over extremely short periods of time and cause molecular alterations of organs and tissues. In the fourth phase (biological), these alterations are transformed into disturbances in cells, organs, and the body as a whole.

These processes undoubtedly occur at any dose of radiation and can be caused not only by radiation exposure, however also by many other factors not related to radiation (Squillaro et al., 2018). The main pathological conditions, including those induced by radiation, are associated with the active initiation of free radical oxidation (FRO) processes. Currently, the mechanisms of urgent nonspecific adaptation at the cellular and subcellular levels after radiation damage have been studied. At the same time, the violation of metabolic processes regulation in cells can act not only as a consequence, but also as a key element of the pathogenetic mechanisms of radiation damage. Exposure to ionizing radiation is characterized by a noticeable activation of FRO processes. It has been established that ionizing radiation contributes to an increase in the concentration of free radicals in various organs and tissues (Shaban et al., 2017). Recent studies emphasize the essential role of free radical processes in the development of occupational pathology.

Recent studies highlight the significant role of reactive oxygen species (ROS) in the mechanisms of development of pathological damage caused by tissue factors (Pedersen et al., 2016). The interaction of phagocytes with harmful particles promotes increased ROS formation, since such particles are resistant to their effects and constantly activate cells. Excessive ROS production can harm the body's own cells, as well as damage nuclear structures due to oxidative modification of proteins, lipids, and nucleic acids (Serrano-Posada et al., 2015). Activation of phagocytes can spontaneously increase, creating conditions for the formation of a vicious circle of inflammation in the affected tissues. Significant consequences of free radicals' effect on living systems include a mutagenic effect and disruption of the structure and function of cells, which is due to the initiation of lipid peroxidation (LPO) processes (Soodaeva et al., 1982). Under normal physiological conditions, LPO is restrained by the antioxidant system, but its functioning can be disrupted under the influence of unfavorable factors (Simioni et al., 2018).

Taking into consideration the importance of the immune system and metabolic processes in the development of pathological condition, as well as their susceptibility to change, high sensitivity and serious consequences in

disrupted, it seems relevant to study their role in the formation of the pathological process in animals under the influence of high doses of ionizing radiation in experimental conditions.

Study Objectives

The aim of the study was to explore biochemical and immunological indicators in experimentally irradiated animals.

Method

To achieve the stated goal, two experimental cycles were conducted on white male Wistar laboratory rats weighing 200 ± 20 grams, which were kept on a standard diet and in normal vivarium conditions. The first experiment involved 20 intact rats, the second – 20 rats irradiated with a dose of 6 Gy. The experiments with rats were completed by the method of partial decapitation under the influence of light ether anesthesia, 14 days after irradiation. In this case, the requirements set forth in the Helsinki Declaration on the humane treatment of animals were strictly observed (World Medical Association, 2002).

The second group animals were exposed to a single irradiation dose of 6 Gy using ^{60}Co gamma rays on the TERAGAM radiotherapeutic device (ISOTREND spol. s.r.o., Czech Republic). Before the irradiation procedure, a thorough topometric and dosimetric adjustment was carried out, including placing the object on the isocentric therapeutic table of the Terasix X-ray simulator (Czech Republic), which is similar in its characteristics and purpose to the table of the gamma irradiator. Data on the cross-section of the pattern of irradiated animals, obtained after output to the display screens, were transmitted to the planning system via a computer network using a digitizer. The PlanW-2000 program calculated isodoses, creating a topometric and dosimetric map indicating the technical parameters and planned irradiation doses.

The animals were exposed to total gamma-irradiation at a dose of 6 Gy once: SSD - 97.2 cm, SAD - 100.0 cm, field 40x40 cm, $t = 352$ sec. (SSD is the distance from the source of ionizing radiation in the apparatus to the conventional center of the irradiated pathological focus; SAD is the distance from the source of ionizing radiation in the apparatus to the closest surface of the irradiated object). During irradiation, the animals were kept in a specially designed cage made of organic glass with isolated cells for each animal.

During the experiment, blood was taken from all animals and the total number of white blood cells (leukocytes) and their subtype, lymphocytes, were counted. For a more detailed analysis of lymphocytes, namely B- and T-lymphocytes and their various subtypes, the immunofluorescence method was used. This method involves staining the cells with special antibodies that "glow" under a fluorescence microscope owing to the fluorochrome attached to them - fluorescein isothiocyanate (FITC). Antibodies that recognize CD3+, CD4+, CD8+ and CD20+ markers on the surface of lymphocytes (these markers indicate belonging to certain subtypes of lymphocytes) were purchased from the American company GALTAG Laboratories. After staining, the cells were examined under a fluorescence microscope, which allowed for a quantitative assessment of the various populations of lymphocytes.

To assess the activity of neutrophils, the nitroblue-tetrazolium test (NBT) was performed and the relative density of insoluble blue formazan granules formed in leukocytes was calculated (Damle et al., 2022). The leukocyte sensitization degree is expressed by the leukocyte migration inhibition reaction with phytohemagglutinin (Artemova, 1973) and the concentration of circulating immune complexes (CIC) (Grinkevich et al., 1981). For quantitative data processing, the immunoregulatory index was determined. In all animals, lipid peroxidation outputs and antioxidant defense enzymes (AOP) were determined in organs and cells. Lymphocytes were isolated from peripheral blood, and homogenates were prepared from the pancreas. The content of diene conjugates (DC) [Gavrilov et al., 1983] and malon dialdehyde (MDA) (Konyuhova et al., 1989), enzyme glutathione reductase (GLR) and glutathione peroxidase (GLP) (Vlasova et al., 1990), catalase (CT) activity (Korolyuk et al., 1988) were determined in them.

The obtained data were subjected to statistical processing using the program "Statgraphics Plus for Windows" (Statpoint Technologies, Inc.). For the analysis, group indicators of summary statistics were calculated, including the arithmetic mean (M) and standard deviation. The reliability of differences was assessed using Student's t-test.

Results and Discussion

The study showed that in experimental animals, exposure to high doses of radiation acted as an immunosuppressive agent, with significant sensitivity of leukocytes and T-lymphocytes and their subpopulations. The number of leukocytes in irradiated animals decreased by 19.0% ($p<0.05$). A decrease in the percentage and absolute number of lymphocytes by 17.62% ($p<0.05$) and 9.05% ($p>0.05$), respectively, was observed (Table 1). As for T-lymphocytes, the following changes were observed: the total number was reduced by approximately 20.25% ($p<0.05$), and the relative number was reduced by 15.36% ($p<0.05$). Accordingly, cells with CD4+ helper cell activity were significantly suppressed: the total number decreased by 21.33% ($p<0.05$), and the relative number decreased by 21.80% ($p<0.05$). The researchers found that the activity on the T-suppressor side was suppressed, with an overall decrease of 14.28% ($p<0.05$), and a relative number of 12.25% ($p>0.05$) (Table 1). Analysis of the study results showed that the absolute and relative number of CD20+ lymphocytes in the blood after irradiation in animals did not change significantly, compared to the control, there was a tendency for these indicators to decrease ($p<0.05$).

It is commonly known that the lymphokine-producing activity of T-lymphocytes reflects the functional activity of the T-system of immunity (Yahyapour et al., 2018). The study showed that irradiated animals showed a decrease in the lymphokine-producing capacity of T-lymphocytes, an increase in the migration index in the leukocyte migration inhibition reaction (LMIC) to PHA by 28.41% compared to the control group ($p<0.05$). In the control group, the ability of cells to produce cytokines that suppress leukocyte migration was expressed. Whereas when exposed to gamma-radiation, cytokine production was apparently significantly lower, thereby exerting an immunosuppressive effect of this factor, which led to the suppression of cytokine synthesis, since the migration index was higher than in intact animals. According to a numerous researcher (Khan et al., 2018, Rak et al., 2015), ionizing radiation causes a decrease in DNA synthesis and the number of cells transformed into blasts under the influence of PHA, a decrease in the production of MIF and the cytotoxic effect. At the same time, ionizing radiation causes a number of interrelated specific and non-specific manifestations. Suppressed CD4+ level and stable CD20+ level indicate the implementation of Th2 immune response, which thereby activates B-cells, evidence of this is the presence of CD20+ concentration at the level of the intact group.

Against the background of suppression of cellular immunity, the result of the immunoregulatory index (IRI) was obtained, a shift to the left towards an insignificant decline of 8.95% ($p>0.05$). A decrease in IRI is characteristic of immunodeficiency states, oncological diseases. Thus, a pathognomonic laboratory manifestation of immune deficiency is almost complete or progressing to the complete absence of T-helpers and a decrease in the immunoregulatory index. A decrease in the immunoregulatory index in our example occurred as a result of a sharp decrease in T-cells with helper activity compared to T-cells with suppressor activity. T-helpers stimulate the population of B-lymphocytes to the process of antibody production. T-helpers make up approximately 55-60% of the total number of circulating T-lymphocytes. Insufficiency of the helper function of T-lymphocytes leads to a decrease in the body's sensitivity to antigen stimulation, contributing to the development of severe infectious complications, the development of possibly malignant neoplasms of radiation origin. Given the complexity of the genesis of changes in immunity during radiation injury to the body, the task was set to experimentally study the reaction of the non-specific phagocytic link of the body during general gamma-irradiation.

In the blood serum of experimental rats, a decrease in the concentration of circulating immune complexes (CIC) by 20.58% was observed compared to the control values ($p<0.05$). Accounting for oxygen-dependent phagocytic killing in the NBT recovery test is an indicator of the phagocytic and metabolic activity of neutrophil granulocytes. The NBT test reflects the final reaction of one of the key enzyme systems responsible for the effector potential of phagocytes. The study showed that the NBT test indicator in experimentally irradiated animals decreased by 34.65% ($p<0.05$), indicating suppression of the functional activity of neutrophils (Table 1).

Thus, in experimental rats irradiated with gamma-radiation, changes were revealed, characterized, first of all, by a decrease in the absolute and percentage number of T-lymphocytes and their subpopulation (CD4+, CD8+), the functional activity of T-lymphocytes, and the body's defense mechanisms in response to exposure to high doses of radiation. A decrease in the number of CD3+ and their subpopulation, which is the immune system's response to the developing pathological process, on the one hand, can be considered as a general physiological reaction of the body in response to stimulation such as stress (Shen et al., 2018). On the other hand, the presence of deteriorations of qualitative and quantitative indicators of cellular and non-specific phagocytic links of immunity, which is a fact of the development of radiation-induced immunodeficiency. The proliferative activity

of T-cells, the quantitative and functional state of the T-system of immunity also decrease. Analysis of the factual material showed that gamma radiation at a dose of 6 Gy has a suppressive effect not only on the T-cell link, but also on the non-specific phagocytic link of immunity, indicating a decrease in the functional and metabolic activity of neutrophils.

Table 1. The results of the study of the immune system under the influence of radiation

Indicators	Control group		Experimental group	
	Abs. number	%	Abs. number	%
WBC, $\times 10^9/l$	6,26 \pm 0,36	-	5,07 \pm 0,39 *	-
Lymphocytes, $\times 10^9/l$	2,61 \pm 0,16	35,78 \pm 2,17	2,15 \pm 0,16 *	32,54 \pm 2,35
T-lymphocytes (CD3+), $\times 10^9/l$	1,58 \pm 0,09	29,55 \pm 1,47	1,26 \pm 0,06 *	25,01 \pm 1,71 *
T-helpers (CD4+), $\times 10^9/l$	0,75 \pm 0,05	18,48 \pm 1,54	0,59 \pm 0,03 *	14,45 \pm 1,04 *
T-suppressors (CD8+), $\times 10^9/l$	0,56 \pm 0,03	11,43 \pm 0,63	0,48 \pm 0,02 *	10,03 \pm 0,51
B-lymphocytes (CD20+) $\times 10^9/l$	0,43 \pm 0,03	6,64 \pm 0,48	0,41 \pm 0,02	6,08 \pm 0,35
IRI (CD4/CD8)	1,34 \pm 0,07	-	1,22 \pm 0,05	-
LMIR	0,88 \pm 0,05	-	1,13 \pm 0,08 *	-
Circulating immune complexes (CIC)	1,36 \pm 0,08	-	1,08 \pm 0,06 *	-
Nitro blue tetrazolium (NBT-test, %)	-	3,78 \pm 0,26	-	2,47 \pm 0,18 **

Note: The difference is significant compared to the control group * - $p < 0,05$; ** - $p < 0,01$.

At the next stage, the effect of high doses of radiation on the formation of lipid peroxidation products DC and MDA in organs and lymphocytes was studied. The results of the study showed (Table 2) that after irradiation, the concentration of DC in peripheral blood lymphocytes in animals exceeded the control values by 62.07% ($p < 0.01$), and in pancreatic homogenate - by 66.19% ($p < 0.01$). And the concentration of MDA was also recorded with an increase in peripheral blood lymphocytes and pancreatic homogenate of experimental rats: 69.23% ($p < 0.01$) and 57.14% ($p < 0.01$), respectively, compared with the control animals.

The results show that exposure to radiation factors activates the oxidation of free radicals, which may be associated with a decrease in the activity of antioxidant enzymes in the studied objects. Activation of lipid peroxidation is based on the excessive generation of active forms of oxygen, exceeding the physiological capabilities of AOS, which arise after the depletion of enzyme systems, as well as a combination of these mechanisms in the case of exposure to a radiation factor, on the one hand, is determined by the massive death of radiosensitive cells of the body and the loss of antioxidants, and on the other hand, by the generation of an active initiator of LPO (Azzam et al., 2012; Shishkina et al., 2015).

Table 2. The results of the study of LPO of the body under the influence of radiation

LPO output	Research object	Control group	Experimental group
DC	Pancreas	0,71 \pm 0,06	1,18 \pm 0,10 **
	Blood lymphocytes	0,29 \pm 0,02	0,47 \pm 0,04 **
MDA	Pancreas	0,21 \pm 0,02	0,33 \pm 0,02 **
	Blood lymphocytes	0,13 \pm 0,01	0,22 \pm 0,02 **

Note: The difference is significant compared to the control group ** - $p < 0,01$.

In the next series of experiments, the effect of high doses of ionizing radiation on the antioxidant system of the pancreas and blood lymphocytes was studied: on the activity of the enzymes catalase, glutathione peroxidase and glutathione reductase. One of the enzymes of the antioxidant defense of the body is catalase, which is involved in the removal of active forms of oxygen, thereby increasing the adaptive response of the body. After irradiation, the suppression of catalase activity in the two studied objects - the pancreas and peripheral blood lymphocytes - remains: 13.74% ($p > 0.05$) and 23.57% ($p < 0.05$), respectively (Table 3).

Table 3. The results of the study of the AOP of the body under the influence of radiation

Enzyme	Research object	Control group	Experimental group
Glutathione reductase	Pancreas	28,15 \pm 2,56	21,34 \pm 1,81 *
	Blood lymphocytes	13,14 \pm 0,97	8,18 \pm 0,63 **
Glutathione peroxidase	Pancreas	158,24 \pm 10,17	138,26 \pm 12,37
	Blood lymphocytes	422,66 \pm 30,42	347,24 \pm 21,75*
Catalase	Pancreas	67,63 \pm 5,22	58,34 \pm 4,36
	Blood lymphocytes	88,22 \pm 6,27	67,43 \pm 4,15 *

Note: The difference is significant compared to the control group * - $p < 0,05$, ** - $p < 0,01$.

An important enzyme of the antioxidant defense of the body is glutathione peroxidase, which protects the body from oxidative damage of any genesis [Matoušková et al., 2018]. GLP catalyzes the reduction of lipid peroxides to the corresponding alcohols and the reduction of hydrogen peroxide to water. In animals exposed to gamma radiation, glutathione peroxidase activity decreased: in the pancreas - by 12.63% ($p > 0.05$) and in blood lymphocytes - by 17.84% ($p < 0.05$). In the reaction of glutathione reductase to irradiation in both studied objects, depression is observed: in the pancreas - 24.19% ($p < 0.05$), in lymphocytes - 37.75% ($p < 0.01$).

Thus, a sharp decrease in the activity of glutathione reductase, catalase and glutathione peroxidase was detected during radiation exposure of the pancreatic homogenate and blood cells studied. Adaptation processes of the body, as is known, also depend on the function of the AOD system. The experimental radiation pathological process is accompanied by a pronounced disruption of the functional activity of the most crucial adaptive and adaptive systems of the body and the accumulation of toxic compounds in tissues that affect their function. The antioxidant system of the cell, tissue and body as a whole ensures the binding and modification of free radicals, preventing the formation and destruction of biomolecules (Barrera, 2012; Mitciov, 2015).

The results of the studies show significant changes in lipid peroxidation and the antioxidant system during radiation stress. Disruption of the functional connections of the catalytic reduction system of glutathione, accompanied by an inhibitory direction of change in the activity of glutathione-dependent enzymes and long-term stress of the links of the antioxidant system, may contribute to a decrease in the antioxidant status of the body, which indicates the need to develop promising methods of adaptive correction in stress of radiation genesis.

Conclusion

Exposure to high doses of radiation causes suppression of the cellular immune system, especially T-lymphocytes and their subpopulations, and adaptive mechanisms of the body of an immune nature. Exposure to ionizing radiation led to a disruption of antioxidant defense mechanisms and the development of lipid hyperperoxidation syndrome.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS Journal belongs to the authors.

Conflicts of Interest

* The authors declare no conflict of interest.

Funding

* This research was funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant No. AP19677010.

Acknowledgements or Notes

* This article was presented as a poster presentation at the International Conference on General Health Sciences (www.icgehes.net) held in Trabzon/Türkiye on May 01-04, 2025.

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Author(s) Information

Oralbek Ilderbayev

L.N. Gumilyov Eurasian National University
010008 Astana, Satpayev 2, Republic of Kazakhstan.
Contact e-mail: oiz5@yandex.ru

Kerim Mutig

I.M. Sechenov First Moscow State Medical University,
119991, 8-2 Trubetskaya str. Moscow, Russian Federation

Gulzhan Ilderbayeva

L.N. Gumilyov Eurasian National University
010008, Astana, Satpayev 2, Republic of Kazakhstan

Darkhan Uzbekov

L.N. Gumilyov Eurasian National University
010008 Astana, Satpayev 2, Republic of Kazakhstan.

Aruzhan Ilderbayeva

University of Duisburg-Essen,
45117 Essen, Germany

Ainur Zhanilova

L.N. Gumilyov Eurasian National University
010008, Astana, Satpayev 2, Republic of Kazakhstan

To cite this article:

Ilderbayev, O., Mutig, K., Ilderbayeva, G., Uzbekov, D., Ilderbayeva, A., & Zhanilova, A. (2025). Acute ^{60}Co Γ -irradiation effect on lipid peroxidation and immune system in experimental animals. *The Eurasia Proceedings of Health, Environment and Life Sciences (EPHELS)*, 17, 27-34.