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Free Radical Pathology of the Body in the Long-term Period under Combined Exposure to Gamma Radiation and Emotional Stress in the Experiment

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ABSTRACT

We have studied the intensity of free radical and antioxidant processes in organs (liver, spleen, thymus, lymph nodes of the small intestine, and adrenal glands) and cells (lymphocytes) in the long-term period after combined exposure to a sublethal dose of γ-radiation (6 Gy) and emotional stress. Combined exposure was followed by accumulation of LPO CD and MDA (conjugated dienes and malonic dialdehyde) products in homogenates of the studied organs. To achieve the goal set, experiments were conducted on 40 outbred white mature male rats randomized into groups. The results of the study indicate major changes in lipid peroxidation and antioxidant system under emotional and radiation stress. Ionizing radiation has a dominating role under combined emotional and radiation aftereffect. This resulted in inhibition of antioxidant protection enzymes and development of dual-oxidative stress in experimental animals. Impaired functional relationships of catalytic redox system of glutathione accompanied by inhibiting orientation of changes in activity of glutathione-dependent enzymes, and prolonged tension of the links of antioxidant system can eventually lead to a decrease in antioxidant status of the body, which indicates the need for the development of advanced methods for adaptive correction.

KEYWORDS
Radiation, emotional stress, free radical processes, antioxidant protection, combined exposure

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Introduction

Free radical oxidation reactions are initiated by the active oxygen configurations leading to chemical modification and destruction of biomolecules. Due to the presence of composite enzyme complexes in the body with specific electron transport prosthetic

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and coenzyme groupings, oxygen recovery process is multistep minimizing the possibility of formation of highly reactive oxygen intermediate compounds. Functional failure of antioxidant system enzymes occurs under the conditions of free radical pathology (Bezruchko et al., 2012; Chou et al., 2007).

A number of pathological conditions including exposures are associated with express initiation of free radical oxidation processes (Moskalev & Strel'tsova, 1987). Today, the mechanisms of the urgent system of nonspecific adaptation at cellular and subcellular levels after radiation injury have been studied. In this case, dysregulation of metabolic processes in cells can be not only a consequence, but the most important element of pathogenetic mechanisms of radiation injury (Musagalieva & Uteshev, 1988). Exposure to ionizing radiation is characterized by a significant activation of free radical oxidation. It has been established that ionizing radiation increases the concentration of free radicals in various organs and tissues (Baraboi & Chebotarev, 1986; Zhuravlev, 1982; Ilderbayev & Dalenov, 2012a; Ilderbayev & Dalenov, 2012b).

The possibility of radiation exposure as a result of practical usage for atomic energy, industrial and medical radiation sources necessitates the study of the effects of radiation on metabolic processes. The risk of radiation exposure increases because of nuclear weapon tests and radiation accidents, e.g. accidents in Chalk River (1952), Border District Mayak (1957), Windscale (1957), Atomic Power Station Svyatoi Lavrentii (1969), Three Mile Island (1979), Chernobyl (1986), and Fukushima-I (2011) (Tapbergenov, 2013). The destructive effect of ionizing radiation is mediated by chain free radical reactions accompanied by LPO activation. Antioxidant system of the body, which condition determines radiation resistance plays the crucial role in the normalization of autoregulation for oxidation-reduction reactions (Abdrakhmanov & Ermakova, 1995; Falt et al., 2003; Hei et al., 2011).

Long-term effects of exposure are mostly the occurrence of leucosis and malignant tumors. It was also found that the effect of radiation on human health may depend on exposure duration: the same radiation dose received over a short period of time causes less damage than the dose received over a long period (Yablokov, 1995).

The investigation of the long-term consequences of ionizing radiation effects on population health are urgent in Kazakhstan. Local radioactive precipitations fell in several areas of this country due to nuclear tests conducted at the Semipalatinsk Nuclear Test Site, and the population of these areas was subjected to internal and external irradiation. People who have received a certain radiation dose, while being at these sites, also face many other everyday difficulties causing emotional distress (Mello et al., 2011).

Despite the large number of experimental and clinical studies, there is no clear idea about the change in free radical oxidation conditions, which indicates the need to study biochemical aspects of adaptation process, in particular, biochemistry of immunocompetent organs. Under physiological conditions, LPO is limited by antioxidant protection, the failure of which may occur at exposures of harmful factors (Rao & Mueller, 1993; Baraboi, Orel & Karnaukh, 1991). Considering the importance of this system in pathological process formation, its role in formation of pathological process in the animals in the long term at combined exposure is of interest to us.

Purpose of the Study

Here we have studied the long-term effects of free radical oxidation in adrenal gland tissues and immunocompetent organs and cells after combined exposure to sublethal dose of γ-radiation (6 Gy) and emotional stress in the experiment.

Materials and Methods

To achieve the goal set, experiments were conducted on 40 outbred white mature male rats (220±20 g) randomized into four groups: group I, control; group II, exposure to emotional stress, group III, exposure to radiation: group IV, combined exposure to emotional stress and radiation.

In groups II and IV emotional stress in the animals was simulated by hanging by the tail, the experiment was ended 1 day after exposure to stress. Group III and IV animals were exposed 90 days before the study to γ-rays at Teragam Co60 γ-therapy apparatus one time at a doze of 6 Gy. Experiments were performed in accordance to the order of the Geneva Convention (1990), and Declaration of Helsinki on Animal's Welfare, under ethical norms of the local ethical committee of the university. Before the exposure, topometry and dosimetry of the rats was performed. To this end, the object was placed on an isocentric therapeutic table of Terasix X-ray simulator (Czech Republic), which is similar to the therapeutic table of the γ-apparatus by its construction and parameters. The images of irradiated animals after displaying were directly input in the planning system using network connection with the computer by digitizer. Isodoses were calculated using planning software PlanW-2000, and topographometric-dosimetric chart with technical characteristics and planned radiation doses was obtained. The animals were exposed to single whole-body γ-radiation in a dose of 6 Gy (one time): 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 conditional center of the irradiated pathologic abnormality: SAD is the distance from the ionizing radiation source in the apparatus to the nearest surface of the irradiated object). During the exposure, the animals were placed in a specially engineered cage made of organic glass with individual compartments for each rat. The products of lipid peroxidation in various organs and cells were determined in all animals. The lymphocytes were obtained from peripheral blood for the study, and homogenates were prepared from the liver, spleen, thymus, lymph nodes of the small intestine and adrenal glands. The levels of conjugated dienes (CD) (Gavrilov, 1983) and malonic dialdehyde (MDA) were evaluated (Konyukhova et al., 1989), as well as activity of the enzyme of glutathione reductase and glutathione peroxidase (Vlasova, 1990), catalase and superoxide dismutase (Korolyuk et al., 1988).

The obtained data were statistically analyzed: differences were estimated using Student's t test (Glantz, 1999; Prise, 2006).

Results and Discussions

As the studies show (Table 1), after emotional stress, CD concentration decreases in peripheral blood lymphocytes from 0,24±0,02 to 0,19±0,01 ($p<0,05$), when there was a significant increase in this value in the other organs: in thymus from 0,47±0,04 to 0,68±0,06 ($p<0,05$), in liver from 0,67±0,06 to 3,34±0,27 ($p<0,001$), in spleen from 1,27±0,15 to 3,65±0,33 ($p<0,001$), in adrenal gland from 1,17±0,12 до 1,49±0,10 ($p<0,05$). In the animals, after radiation aftereffect, CD concentration in peripheral blood lymphocytes has significantly (nearly 1.33 times) and in lymph nodes of the small intestine (1.39 times) exceeded the control values (group I, $p <0,05$). And in other examined organs of group III animals: liver, spleen, thymus and adrenal gland, doubtful change was revealed, but there was a trend towards increase in CD product concentration ($p>0,05$). According to the study, we can say that activation of lipid peroxidation is more pronounced after emotional stress than in the long-term period of gamma radiation aftereffect.

In the animals, after combined exposure, CD concentration in peripheral blood lymphocytes has significantly (almost 1.79 times) exceeded the control values (p<0,01). In the animals of group IV subjected to combined exposure, CD level in adrenal glands increased from 1,17±0,12 до 1,69±0,14 (p<0,05), and in blood lymphocytes it increased from 0,24±0,02 to 0,43±0,05, i.e. 1.79 times as compared with the value of group I (p<0,05). The study of primary products of lipid peroxidation in liver and lymph nodes showed that the content of conjugated dienes in liver increased from 0,67±0,06 to 3,68±0,31 (p<0,001), approximately 81.79%, in lymph nodes from 0,33±0,03 до 0,59±0,05 (p<0,01), approximately 78.79% (p<0,01).

CD concentration in spleen in the animals of group IV is 1.76 times increased (p<0,05), in thymus – almost 1.21 times increased (p<0,05). The results show that under the exposure of emotional and radiation factors, free radical oxidation activates, perhaps this is due to decreased activity of antioxidant enzymes in most studied organs in the long-term period of aftereffect of gamma radiation. As is known, lipid peroxidation activation is based on excessive generation of active oxygen configurations exceeding physiological capabilities of antioxidant systems, coming after depletion of enzyme systems, as well as a combination of these mechanisms in case of the effect of radiation factor, on the one hand determined by massive loss of radiosensitive cells of the body and loss of antioxidants, and on the other hand –active generation of LPO initiators (Agadzhanyan, Baevsky & Berseneva, 2006; Meerson & Pshennikova, 1988; Richardson, 2009; Usenova, Zhetpisbaev & Saidakhmetova, 2006).

Table 1. Content of CD and MDA in organs and blood lymphocytes in the long-term period under combined exposure to gamma radiation (6 Gy) and emotional stress, M±m,

	The object of research	Group I, control	Group II, exposure to emotional stress	Group III, exposure to gamma radiation	Group IV, combined exposure to emotional stress and gamma radiation
CD	Liver	0,67±0,06	3,34±0,27 ***	0,73±0,06	3,68±0,31***
	Spleen	1,27±0,15	3,65±0,33 ***	1,33±0,11	2,24±0,20 *
	Thymus	0,47±0,04	0,68±0,06 *	0,49±0,03	0,57±0,03 *
	Adrenal glands	1,17±0,12	1,49±0,10 *	1,23±0,11	1,69±0,14 *
	Lymph nodes of small intestine	0,33±0,03	0,41±0,03	0,46±0,03 *	0,59±0,05 **
	Lymphocytes	0,24±0,02	0,19±0,01 *	0,32±0,02 *	0,43±0,05 *
MDA	Liver	0,15±0,01	0,35±0,03 ***	0,14±0,02	0,19±0,02
	Spleen	0,31±0,03	0,28±0,02	0,41±0,03 *	0,78±0,08***
	Thymus	0,17±0,01	0,22±0,02 *	0,22±0,01 *	0,49±0,05***
	Adrenal glands	0,21±0,02	0,26±0,01	0,38±0,02 **	0,61±0,05***
	Lymph nodes of small intestine	0,05±0,005	0,09±0,008 **	0,09±0,007**	0,14±0,01***
	Lymphocytes	0,07±0,006	0,06±0,005	0,12±0,01 **	0,21±0,02***

Note: Differences with the control group are valid: *-p<0,05, ** - p<0,01, *** - p<0,001.

At the next step, we have studied the effect of emotional stress on formation of the end product of MDA lipid peroxidation in organs and lymphocytes. MDA content in lymph nodes was 1,80 times (p<0,01), in thymus 1,29 times (p<0,05) and in liver 2,33

times higher ($p<0,05$) than in the control animals. Under these conditions, MDA level in spleen, adrenal glands and peripheral blood lymphocytes did not change.

The studies have shown that pathological condition caused by gamma radiation was accompanied by an increase in MDA in peripheral blood lymphocytes and thymus of rats: 1,71 and 1,29 times ($p<0,05$), respectively, compared with the control animals. However, in liver, doubtful decrease in MDA level under the effect of gamma radiation ($p>0,05$) was noted. The effect of experimental irradiation of rats in the long-term period is accompanied by accumulation of MDA in spleen, adrenal glands, lymph nodes of the small intestine: 1,32 times ($p<0,05$), 1,81 times ($p<0,01$) and 1,80 times ($p<0,05$), respectively, compared with the control group.

Combined exposure of the studied factors on formation of MDA of main end LPO product in organs and lymphocytes has shown the following – each object under study has shown a significant increase in MDA level: in lymphocytes –200,0% ($p<0,001$), in spleen –151,61% ($p<0,001$), in lymph nodes –180,0% ($p<0,001$), in adrenal gland – 190,47% ($p<0,05$), in thymus – 188,23% ($p< 0,001$) compared with the control group (group I). Doubtful increase in MDA level under the effect of two factors ($p>0,05$) was noted in liver. Comparative analysis of MDA content in various tissues of the animals under combined exposure to these agents has shown that increase in the levels of lipid peroxidation products in the long-term period was characteristic also for tissues with higher (spleen, liver) and lower proliferative and metabolic activity (adrenal glands).

In the next series we have studied the effect of separate and combined exposure to emotional stress and ionizing radiation in the long-term on antioxidant system in organs and blood lymphocytes. Changes in LPO system subject can not be without change in AOS activity to their relationship. As a result of emotional stress, increased activity of superoxide dismutase in spleen was revealed – 1,29 times ($p<0,05$), and in blood lymphocytes – 1,3 times ($p<0,05$). In these animals, activity of catalase, glutathione peroxidase and glutathione reductase in blood lymphocytes and lymph node homogenates was significantly higher than the control values: catalase – in lymphocytes 1,33 times ($p<0,05$), in lymph nodes 1,49 times ($p<0,05$); glutathione peroxidase – in lymphocytes 1,28 times, in lymph nodes – 1,29 times ($p<0,05$); glutathione – in lymphocytes 1,39 times ($p<0,05$), in lymph nodes – 1,59 times ($p<0,05$). Increase in activity of these enzymes indicates the increase in concentration of active oxygen configurations and their peroxide compounds under emotional stress, causing damage to the integrity of tissue cells. These studies show that under emotional stress, activities of the above enzymes in liver, spleen, thymus and adrenal glands were not significantly changed, but tend to increase ($p>0,05$).

At the next stage, we have studied the effect of gamma radiation (Table 2) in the long-term period on activity of enzymes of SOD, catalase, glutathione peroxidase and glutathione reductase in homogenates of organs and cells. SOD activity in lymphocytes, lymph nodes, thymus and spleen was inhibited: in lymphocytes 34,56% ($p<0,05$), in lymph nodes 26,63% ($p<0,05$), in thymus 30,51% ($p<0,05$), in spleen 37,13% ($p<0,05$). SOD activity in liver and adrenal glands remained at the level of the control groups.

One of enzymes of AOP (antioxidation protection) is catalase involved in destruction of active oxygen configurations, thereby increasing the adaptive response of the body. In the long-term period, suppression of catalase activity in liver, spleen, thymus, lymph nodes is maintained after radiation: 1,48 times ($p<0,05$), 2,22 times ($p<0,05$), 2,04 times ($p<0,05$), 1,55 times ($p<0,05$), respectively. An important enzyme of AOP is glutathione peroxidase, protecting the body from oxidative damage. Glutathione peroxidase catalyzes recovery of lipid peroxides to the corresponding alcohols and recovery of hydrogen peroxide to water. Thus, inhibition of

hyperlipoproteinemia enzyme activity was noted in all the studied objects; a significant decrease was revealed in lymphocytes – 1,32 times (p<0,05) and in spleen – 1,53 times (p<0,05).

Table 2. The effect of emotional and radiation factors on activity of AOP enzymes

	The object of research	Group I, control	Group II, exposure to emotional stress	Group III, exposure to gamma radiation	Group IV, combined exposure to emotional stress and gamma radiation
Glutathione reductase	Liver	25,44±2,42	28,98±2,45	16,18±1,54 *	22,66±2,12
	Spleen	37,33±3,81	44,35±4,68	21,66±2,21 *	26,22±2,53 *
	Thymus	31,44±3,04	34,87±3,51	23,55±2,42 *	23,65±2,42 *
	Adrenal glands	24,33±2,52	26,58±2,01	21,17±2,02	26,55±2,53
	Lymph nodes of small intestine	27,29±2,45	43,42±4,21 *	19,57±2,37 *	21,21±2,03
	Lymphocytes	8,11±0,78	11,28±0,97 *	4,37±0,31 **	11,54±0,98 *
Glutathione peroxidase	Liver	177,33±15,55	273,14±22,35 *	156,78±16,13	126,63±10,53 *
	Spleen	267,55±24,66	255,58±23,67	174,53±13,17 *	149,38±13,28 **
	Thymus	128,88±13,02	148,45±12,04	110,10±9,98	89,66±9,88 *
	Adrenal glands	178,65±15,63	363,58±27,32 ***	151,01±13,27	183,87±16,56
	Lymph nodes of small intestine	234,48±20,51	302,54±25,53*	202,33±17,64	183,18±14,11 *
	Lymphocytes	443,02±40,12	567,22±41,14 *	334,12±28,11 *	216,66±24,67**
Catalase	Liver	76,55±5,57	81,25±7,67	51,65±4,66 *	42,66±4,05 **
	Spleen	61,36±5,56	73,54±8,22	27,66±2,32 ***	37,69±3,22 *
	Thymus	55,66±4,87	64,77±5,48	27,27±2,12 ***	31,38±2,98 **
	Adrenal glands	63,56±7,11	70,36±5,44	53,09±4,32	48,22±3,88
	Lymph nodes of small intestine	52,44±4,99	78,28±7,07 *	33,78±3,17 *	34,27±3,13 *
	Lymphocytes	91,33±10,05	121,43±11,05 *	81,38±6,25	67,39±3,36 *
SOD	Liver	64,35±5,65	80,66±7,33	55,11±4,52	46,52±3,86 *
	Spleen	67,33±6,12	86,87±7,05 *	42,33±3,27 *	35,64±3,43 **
	Thymus	51,32±5,04	54,66±4,85	35,66±3,21 *	32,27±3,01 *
	Adrenal glands	43,87±4,11	40,99±4,11	43,02±4,12	46,66±4,05
	Lymph nodes of small intestine	28,65±2,21	34,87±3,11	21,02±2,10 *	28,54±2,75
	Lymphocytes	47,45±4,34	61,68±5,18 *	31,05±3,12 *	35,52±3,45 *

Note: Differences with the control group are valid: *-p<0,05, ** - p<0,01, *** - p<0,001.

The animals received gamma radiation, have shown reduced activity of glutathione reductase in the long-term period: in liver 1,57 times (p<0,05), in spleen 1,72 times (p<0,05), in thymus 1,34 times (p<0,05), in lymph nodes 1,39 times

($p<0,05$), in lymphocytes 1,86 times ($p<0,01$). These data are again confirmed by the results obtained in the study of lipid peroxidation products, where lipid peroxidation product concentration was significantly increased.

In the next series, we have studied the effect of combined exposure to emotional stress and ionizing radiation on AOP in organs and blood lymphocytes. In these animals, activity of enzymes of SOD, catalase and glutathione reductase in almost all studied objects was inhibited. The animals receiving combined exposure to gamma radiation and emotional stress, activity of superoxide dismutase decreased in the long-term period: in liver 1,38 times ($p<0,05$), in spleen 1,89 times ($p<0,05$), in thymus 1,59 times ($p<0,05$), in lymphocytes 1,34 times ($p<0,05$). The same situation was observed in the study of activity of catalase and glutathione peroxidase, i.e. combined exposure to the studied factors on AOP enzyme activity in organs and lymphocytes has shown a decrease in activity of catalase and glutathione peroxidase.

Conclusion

Thus, it was found that in the studied homogenates of organs and cells with combined emotional and radiation stress exposure, activity of superoxide dismutase, catalase and glutathione peroxidase is dramatically decreased. Adaptive processes of the body are known to be heavily dependent on the function of antioxidant protection system. Experimental emotional and radiation pathological process is accompanied by severe impaired functional activity of the most important adaptive systems of the body and accumulation of toxic compounds in the tissues that affect their function. Antioxidant system of the cell, tissues and body as a whole provides binding and modification of free radicals, preventing formation and destruction of biomolecules (Baryshnikov & Baryshnikov, 1997; Kurlyandskii & Filova, 2002).

The results of the study indicate major changes in lipid peroxidation and antioxidant system under emotional and radiation stress. Impaired functional relationships of catalytic redox system of glutathione accompanied by inhibiting orientation of changes in activity of glutathione-dependent enzymes, and prolonged tension of the links of antioxidant system can eventually lead to a decrease in antioxidant status of the body, which indicates the need for the development of advanced methods for adaptive correction. The results of the work allowed us to make the following conclusions: 1. Emotional stress activates lipid peroxidation and reduced antioxidant protection in immunocompetent organs and cells. 2. In the long-term period of gamma radiation aftereffect, inhibitory effect of the radiation factor on antioxidant protection maintains. 3. Ionizing radiation, combined with emotional stress in the long-term period has a more pronounced effect with formation of lipid hyper-peroxidation syndrome. 4. Ionizing radiation was the dominant agent in the developed free radical pathology under combined exposure to two factors.

Disclosure statement

No potential conflict of interest was reported by the authors.

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