Study of immune status and the role of free - radical oxidation in organs and cells under the influence of gamma - radiation in the remote period

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Abstract. We studied the intensity of free radical processes in organs (liver, spleen, thymus, lymph nodes of the small intestine, and adrenal glands) and cells (lymphocytes) and immunity system in the delayed period to a sublethal dose of γ-radiation (6 Gy). To investigate the immunity system and the lipid peroxidation of laboratory animals (white rats) in two series of experiments. The overall number of white mature rats was equal to 30 with body mass varying from 220 to 250 grams. All rats were divided into 2 groups: I – controls, II group – animals exposed to radiation. The animals of the II series were exposed to γ-rays Co^{60} in a Teragam γ-therapy apparatus. Effect to radiation was accompanied by accumulation of LPO products DC and MD in homogenates of the studied organs. There is noted the reduction of main immunity exponents, reducing of T-lymphocytes count, suppression of nonspecific phagocytic organism defense and increase of CIC in blood serum.
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Introduction

One of the basic but nonspecific radiation effects, on living body is activation of free-radical process on the back of which intensify lipid peroxidation. Radio ecological feature of regions of CAPP accident and territories with increased radiation background in the Komi Republic is essentially differs chemically of radionuclides and action time of radiation factor. The same type changes of lipids and ferment strength of dehydration in tissue of wild gnawing animals were founded while the degree of manifestation is essentially higher than by animals from accident zone. For eight years after the accident in issues of murine rodents is not marked full rehabilitation on cellular level. System state of regulation of lipid peroxidation (LP) and processes of dehydration in wild rodent organs and their high sensibility to chronic action of radiation may form the basis of early prediction of biological effects and long-term effects of radiation contamination in native habitat. [1].

The leucosis and the malignant tumors beginning are, more often, the delayed radiation actions. It also has been cleared out, that radiation effect upon the human health is able to be depended on the exposure duration: one and the same dose of the ionizing radiation, having received for the short period of time, is being caused the less radiation injuries, than the dose of the ionizing radiation, having received for the prolonged period [2].

The ionizing radiations exposure delayed consequences researches upon the population health are the most urgent and the most actual in Kazakhstan. It 3s being defined by the fact, that the considerable part of the Republic's territory, first of all, have been subjected by the local radioactive deposits and by the local nuclear fallout, as a result of the nuclear tests operation at the Semipalatinsk Testing Area, and the residential population, having lived on all these above – mentioned territories, have also been subjected by, as the internal, well as the external radiations exposure [3].

The biological radiation effect is, usually, consisted in the further atoms' and molecules' ionization and the excitation just in the human organs and the tissues with the subsequent highly radioactive radicals and the peroxides, and also super oxides formation. So, the first 3 phases are, usually, being proceeded at the molecular level just for the negligibly small periods of time, and they are being caused the molecules' chemical changes just in the human organs and the tissues. Thus, all these changes are being transformed, and they are being converted into the subsequent abnormalities just in the cells, in human organs, and also in the human organism, as a whole, during the 4-th phase (e.g. the biological one). It goes without saying, the above – indicated processes are being taken their place at the every radiation dose exposure, and they are able to be conditioned not only by the exposure to the radiation, but and the many other non- radiation factors action [4]. In the basis of a

number of the pathological states, including the exposure to the radiation, are being connected with the expressed processes initiative of the free radical oxidation. Thus, at present, the non-specific adaptation just at the cellular and the sub-cellular levels after the radiation damage the urgent system mechanism have already been studied. For all this, the metabolic processes regulation violation just in the cells is able to be not only the consequence of it, but also, it is the most significant link of the radiation damage pathogenetic mechanism [5].

In spite of a large number of experimental and clinical researches, there is no clear idea of changes of free-radical oxidation condition that testify of study necessity of biochemical aspects of adaptive process, in particular the biochemistry of immunocompetent organs. In physiologic conditions the LP is limited to antioxidant defense which failure can be on exposure of hazard [6, 7].

One of the most radiosensitive functions of organism and animals is immune responsiveness. It's changes can be identify by objective methods long before the incipiency of clinical symptoms of damage and under the action of different irradiation doses including small doses. The attribute of radiation effects is the long-term damage sustaining in sporadic components of immune system and connected with it long-term effects and complications which are evident with premature senility, fast progression of chronic illness of internal and progression of malignant neoplasm [8, 9].

The authors state that radiation-exposed subjects demonstrating CRS showed an activation of barrier anti-oxidative stress mechanisms at late periods after radiation exposure, apparently in response to a more severe radiation damage than subjects exposed to similar radiation doses but not demonstrating CRS. Finally, the persistence of chromosome aberrations and somatic mutations in the CRS cohort is indicative of an exhaustion of the anti-oxidative stress mechanisms responding for so many years after the exposure, leading to genomic instability [10].

As the ionizing radiation so and peroxidation are biologically significant phenomena effecting on the living system. Their correlation composes the implication of process of radiation damage of cell, organism and underlie the various mechanisms of living system reaction on radiation. At the present time the most extensively studied early effects, which occurs in fast proliferous tissues. At the same time the radiation effect on biochemical reactions which are passing in cells of different organs in remote time after the radiation is deficiently studied.

Taking into account the importance of antioxidant and immune system in formation of pathologic process we have an interest in their role in pathologic process formation by irradiated animals in remote period.

Research objective: Study immune system and the role of FRO in immunocompetent organs and cells of animal body in remote periods after the effect of external total ionizing gamma irradiation in the dose 6 grey.

Material and methods

To investigate the immune system and the lipid peroxidation was used laboratory animals (white rats) in two series. The overall number of white mature rats was equal to 30 with body mass varying from 220 to 250 grams. All rats were divided into 2 groups: I – controls (n=15), II group – animals exposed to radiation (n=15). The animals of the II series were exposed to γ-rays Co^{60} in a Teragam γ-therapy apparatus. We have carried out the animals´ topometric and dosimetric preparation on the «Terasix» X - ray photography simulator, which is provided the correct bringing to the planned dose, before to conduct the irradiations.

To this end, the object was placed on an isocentric therapeutic table of Terasix X-ray simulator, 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 electronic tablet. Isodoses were calculated using planning software Plan W-2000, and the image with radiotherapy plan with technical characteristics and planning radiation doses was obtained. The animals were exposed to single whole-body radiation in a dose of 6 Gy. During the exposure, animals were placed in a specially engineered cage made of organic glass with individual compartments for each rat. In the II group animals were examined 90 days after irradiation. This kind of work with the experimental animals has been conducted, in accordance with the principles of the Helsinki Declaration of the World Medical Association on the humane treatment with the animals. The study protocol was approved by the Local Ethics Committee of the Semey State Medical University, Semey, Kazakhstan with the number of No4 dated January, 2014.

The lipids peroxidation state has been defined just in the various organs and the cells at all the animals. So, the lympocytes have been singled out just from the peripheral blood, and the homogenates have been prepared just from the liver, the spleen, the thymus gland, the lymph nodes of the small intestine and the adrenal glands, which are needed for the necessary investigation.

Primary products of LPO metabolism – CD (conjugateddienes) were formed due to the migration of double bonds of lipidacidacils, with 2 or 3 double bonds causing changes in phospholipids' membranes. Secondary product of PLO's metabolism is MD which form in the destruction of the hydroperoxides lipids and phospholipids.

The immune status has been estimated on the basis of the content study in the lymphocytes´ peripheral blood and their subpopulation with the phenotypes: CD3+, CD4+, CD8+, CD19+ by the method of the immunofluorescent staining of the cells with the antibodies use, having conjugated with the FITC (Fluorescein Isothyocyanate) (the "CALTAG Laboratories" Company, the USA), having adapted, exactly, for the rats´ analyses. The neutrophils´ phagocytal activity (PHA) definition, the CIC (the circulating immune complexes), ITML (the inhibition test of the migration of leukocytes) number have been conducted.

The immunological and biochemical indicators have been defined in the all above - mentioned groups with the statistical processing and with the Student´s criteria calculation carrying out [11].

Results

Research is approved that blood leucocytes of intact animals registered in the limit 6,48±0,51x10^9/l ($p<0,05$). At that, specify rising as percentage so and absolute number of lymphocytes on 19,06% ($p<0,05$) and 18,13% ($p>0,05$). Total amount CD3+ is reduced on 63,70% ($p<0,001$). Both exponents on CD4+ marked authentic reduction: absolute number reduced on 45,71% ($p<0,001$), percentage number on 41,95% ($p<0,001$) (Table 1). After the irradiation, in remote period, educed authentic reduction of absolute and percentage number of CD8+: absolute number on 40,82% ($p<0,001$) and percentage on 44,71% ($p<0,001$). Given change causing the reduction of immunoregulatory balance which average composed 1,29±0,11 and it's lower than in control 1,44±0,1. On the part of CD19+ there is marked rising of both exponents: absolute number on 30,61% ($p<0,001$), percentage on 38,62% ($p<0,01$).

It is known that lymphokine-producing activity of T-lymphocytes reflects functional activity of T-system if immunity. Research is approved that marked reduction of lymphokine-producing ability of T-lymphocytes, rising of migration risk in ITML on FGA from c 0,79±0,04 in intact group to 1,48±0,13 in experiment group ($p<0,001$). Probably, in control group expressed cell ability to produce cytokines repressing leucocytes migration, while in radiation effects in remote period the producing of cytokines was much less because the migration index composed more than at intact animals.

Table 1. Immune system exponents by irradiation animal with γ-radiation, M±m

Exponents		Control animals	Exposed animals
Leucocytes, × 10^9/l		6,48 ± 0,51	4,96 ± 0,42 *
Lymphocyte, × 10^9/l		2,76 ± 0,12	3,41 ± 0,29 *
Lymphocyte, %		39,02 ± 3,23	47,66 ± 4,25
CD3+	Abs.n.°	1,46 ± 0,10	0,62 ± 0,04 ***
	%	31,82 ± 2,41	11,55 ± 1,07 ***
CD4+	Abs.n.	0,70 ± 0,04	0,38 ± 0,04 ***
	%	20,93 ± 1,41	12,15 ± 1,19 ***
CD8+	Abs.n.	0,49 ± 0,02	0,29 ± 0,02 ***
	%	11,25 ± 0,98	6,22 ± 0,63 ***
CD19+	Abs.n.°	0,34 ± 0,02	0,49 ± 0,03 ***
	%	7,23 ± 0,68	11,78 ± 1,08 **
CIC, y.e.		1,26 ± 0,11	1,62 ± 0,12 *
ITML		0,79 ± 0,04	1,48 ± 0,13 ***
Phagocytosis, %		36,17 ± 2,52	22,34 ± 2,02 ***
Cytophagous number		1,59 ± 0,14	1,09 ± 0,09 **
NTT-test, %		4,87 ± 0,41	4,09 ± 0,33

Note: statistic change is significantly in comparison with intact control * - $p<0,05$; ** - $p<0,01$; *** - $p<0,001$. ° - absolute cells number ×10^9/l.

Authentic reduction of phagocytic activity of blood cells on 38,24% ($p<0,001$) by irradiated animals in remote period was detected. List result of latex average number engaged with one phagocyte in irradiated animals group has average registered 1,09±0,09 and it is authentically lower of intact rats exponent ($p<0,01$). There is reduction of CIC concentration in 2,3 times by irradiated rats ($p<0,001$). There are not detected material changes on the part of NTT test in II group. There is reducing trend that indicates decreased activity of neutrophils.

It is known, that free – radical peroxidation of endogenous connectors may reduce to autoimmune antigen becoming. When dealing with albumen of low molecular weight endogenous compounds there may be appearance of conjugated antigen causative antiself organism response. [12]. In this context, we studied DC and MD sustentation in adenoid tissue, lymphocytes and in other organs (Table 2). By the conducted researches it is established that pathological process radiation-induced has accompinied with DC increase in blood lymphocytes and small intestine lymph gland of rats: 1,3 times ($p<0,05$) and 1,4 times ($p<0,01$) in comparison with control animals. There are no detected material changes on the part of hepar, adrenal, thymus, spleen in II group (there is tendention to increase, $p>0,05$).

On the next stage, we studied radiation effect on appearance of end product LP MD in organs and lymphocytes in remote period. Authentic rising of sustentation of given exponent at all under consideration objects except hepar: in spleen 1,3 time, ($p<0,05$), in thymus 1,29 time ($p<0,01$), in adrenals 1,8 time ($p<0,001$), in lymph nodes of small intestine 1,8 time ($p<0,001$) and in blood lymphocytes 1,7 time ($p<0,001$).

Table 2. DC and MD sustentation in organs and blood lymphocytes under the influence of γ-radiation, M±m.

Object	I group Control animals	II group Exposed animals	I group Control animals	II group Exposed animals
	DC (conditional units)		MD (measurement unit – mole/mgr)	
Liver	0,67±0,06	0,73±0,06	0,15±0,01	0,16±0,02
Spleen	1,27±0,15	1,33±0,11	0,31±0,03	0,41±0,03 *
Lymph nodes of small intestine	0,33±0,03	0,46±0,03 **	0,05±0,005	0,09±0,007 ***
Thymus	0,47±0,04	0,49±0,03	0,17±0,01	0,22±0,01 **
Adrenal glands	1,17±0,12	1,23±0,11	0,21±0,02	0,38±0,02 ***
Blood lymphocytes	0,24±0,02	0,32±0,02 *	0,07±0,006	0,12±0,01 ***
Note: statistic change is significant in comparison with intact control * - p<0,05; ** - p<0,01; *** - p<0,001.				

This changes may be explained by chronicity of experimental animals oxidative stress put to irradiating, persisting with increased production of free radicals in tissues, subjected to reparative processes and regeneration, depressing antioxidant mechanisms in consequence of long-term exposure of oxidative stress hostilities. One of the reason of prolonged course of oxidative processes distortion in adrenals may be rising of their functional activity at late after the irradiation because catecholamines, in particular adrenaline, have a knack to contribute in chain free-radical processes in tissues [13].

As opposed to the said tissues in hepar, there were material changes. MD sustentation was at the level of intact animals exponent (there is the tendency to rise). Given fact show that there are big compensation abilities of hepatic tissues which enables abnormalities arresting of free-radical processes induced by the effect of ionized irradiation.

In such a manner, at the irradiated rats in the remote period were revealed decrease in the main indicators of immunity, the quantity of T-lymphocytes, a supressiya of nonspecific phagocytic protection of an organism defense. There was CYC increase in blood serum. Perhaps, it was the result of uprising of organism autoimmune response on conjugated antigen. After the radiation effect, in remote period, there were abnormalities in the peroxidation process of lipids in mainly under investigation immunocompetent organs and tissues.

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