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REPARATIVE REGENERATION OF THYMUS UNDER THE EXPOSURE TO IONIZING RADIATION: HISTOMORPHOMETRIC AND IMMUNOMORPHOLOGICAL STUDY

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Abstract

The aim of this study was to evaluate the time-related changes in histomorphometric and immunomorphological parameters of rat thymus under a single ionizing radiation exposure in sublethal dose of 5 Gray on the background of correction by parenteral administration of xenogenic cerebrospinal fluid.

An experiment with radiation injury of rats at a dose of 5 Gray without correction and with parenteral administration of xenogenic cerebrospinal fluid was carried out. The structural organization of the thymus was assessed by descriptive histological analysis, histomorphometric and immunohistochemical methods (Ki-67 and CD68 markers) followed by statistical analysis of the results.

The revealed changes in structural organization of thymus after radiation injury has shown significant morphological transformations of both histological and cytological structural arrangements of the organ observed on 3rd and 7th days after irradiation, and with the restoration of cell population up to the 30th day of the experiment. A single injection of the cerebrospinal fluid at a dose of 10 mL/kg has excessive and short effect, resulting in aggravation of reactive changes in microvasculature during the early postradiation period followed by the peak of restoration process on the 14th day of the experiment. Repeated administrations of cerebrospinal fluid in a dose of 2 mL/kg result in a peak of intrathymic proliferation, elimination of cells with signs of destruction, restoration of the ratio of structural-functional zones and their cellular composition on the 7th day after irradiation with a constant reparative processes on the 14th and 30th days of the experiment.

Reparative regeneration of the thymus in postradiation period without correction is characterized by the two-wave kinetics with recession on the 3rd and 14th days and relative restoration on the 7th and 30th days after irradiation. Parenteral administration of cerebrospinal fluid has a pronounced effect on thymus histoarchitectonics during the postradiation period. Revealed changes are linear and are aimed at restoring the histological and cytological structure of the thymus, largely depending on the dose and frequency of cerebrospinal fluid administration.

Keywords: *morphology, thymus, rat, ionizing radiation, cerebrospinal fluid.*

Introduction

Thymus as a central organ of immunogenesis plays a key role in the formation of cell-mediated immunity and is highly susceptible to neurohumoral regulatory effects [*Pearse G, 2006*]. Rising anthropogenic pollution increasingly leads to development of immunopathological states affecting the thymus in particular, which structural organization of parenchymal and stromal-vascular components is associated closely with the formation of immunological competence of an organism [*Mitin A et al., 2012*]. Ionizing radiation should be considered as one of these factors with a pronounced negative effect on thymus. According to literature data, the effect of ionizing radiation leads to a state of immune suppression which is evident in structural and functional reorganization of thymus [*Mitin A et al., 2012; Bibik E et al., 2014*]. Morphological transformations of central and peripheral organs of immu-

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nogenesis, including the thymus, in normal state [*Yarilin A, 1999; Pearse G, 2006; Moroz G, 2009*] and after ionizing radiation exposure [*Yarilin A, 1997; Mitin A, 2012*] are widely discussed in literature. The time-related structural changes of the thymus in response to different types and doses of ionizing radiation, including general and local irradiation, was demonstrated on various experimental animals [*Park H et al, 2006; Ohi H et al,. 2007; Li J et al., 2012*]. However, available published data do not allow predicting accurately the changes of histopathological reactions in the thymus exposured to a certain dose of radiation within a particular experimental model. The time-related changes of various immunohistochemical markers and histomorphometric parameters should also be considered as one of poorly studied questions within this field.

On the other hand, issues of thymus morphogenesis under the exposure to exogenous factors involve the fundamental problem of the interaction of nervous, endocrine and immune systems that encourage researchers to determine the mechanisms of supra-system regulation and methods of influence on such regulation [*Webster J et al., 2002; Jara L et al., 2006; Moroz G, Kriventsov M, 2013*]. In this context, the most promising approaches are believed to be based on the study of natural processes in the organism, as well as on the search for the medicines of natural origin that could have an impact on different levels of regulation. Concurrently, there is a need for new, highly efficient and safe method of protection against gamma irradiation or subsidence of its negative consequences in medicine. For that matter, a promising raw material for biogenic medicine with radioprotective properties is cerebrospinal fluid (CSF). Theoretical and experimental basis for use of xenogenic CSF as a medicinal raw material was made during first half of the XX century, when attempts to use the autologous, allogenic or heterogenic CSF in various clinical and experimental models, including models of acute radiation injury, were made [*Kuprijanov S, Mamieva M 1968; Tkach V et al, 1983*].

Combining the two pressing problems, the search for highly effective medicines with radioprotective properties and evaluation of inter-system interaction mechanisms in the aspect of physiological role of the CSF, suggests the idea of theoretical possibility of studying fundamental issues of influence the neuroendocrine signals on the functional state of immune system through the "transfer" of substrate possessing a specific activity from one organism to another. Additionally, it is possible to study the CSF as a medicinal raw material by identifying potential safety profile and the range of effects on target organs in the experiment.

Thus, given the urgency of studying radiation damage to the organs of the immune system and searching for new highly effective radioprotective agents, including those of biological origin, the aim of this experimental research was to study the time-related changes of histomorphometric and immunomorphological parameters of rat thymus under a single exposure to ionizing radiation in a sublethal dose of 5 Gray on the background of correction using parenteral administration of xenogenic cerebrospinal fluid.

Materials and methods

Drawing of xenogenic cerebrospinal fluid. Cerebrospinal fluid was drawn in vivo by suboccipital puncture. Cows were used as donor animals. The obtained CSF was on-site evaluated for the criteria of color, transparency, and presence of visible impurities. CSF was supposed to be a colorless or slightly yellowish, clear liquid, without any visible inclusions. Otherwise, the obtained CSF was rejected. Subsequently, CSF in plastic tubes was subjected to ultrafast freezing by immersion in liquid nitrogen at a temperature of -196ºC in Dewar vessel. Immediately prior to administration, CSF was thawed at room temperature. The method of in vivo drawing, preservation and subsequent use of xenogenic CSF is described in the literature [*Tkach V, 1983*].

Experimental model of ionizing radiation exposure. Experimental animals were exposed to a single ionizing gamma irradiation at a dose of 5 Gray using Clinac 2100 linear accelerator (Varian, USA) on the basis of the Crimean Republican Institution "Oncological Clinic Dispensary" (Simferopol). The following ionizing radiation parameters were used: the working energy of the

√ linear accelerator – 6 MeV,
√ exposure time – 50 seconds,
√ single dose – 5 *Gray*, f
√ ield size – 40 cm^2,
√ and penetration depth – 2.5 *cm*.

During the irradiation the rats were in the free mode of movement within a specially designed box with an area of 40 cm^2, which ensured the uniform effect of ionizing radiation on animals. Among the above mentioned parameters, the key parameter is a dose of radiation equal to 5 Gray, which is widely used to simulate the bone marrow form of acute radiation sickness in rats. At the same time, the selected dose does not reach the LD50 for rats (according to various literature data, ranging from 6 to 8.5 *Gray*) [*Williams J et al., 2010*].

Material of experimental study. The experimental study was performed on 84 male juvenile Wistar rats (age 3-3.5 months, body weight 150-170 *g*). According to the aim of the study, the experiment was divided into two series of experiments (S1 and S2), each of which included experimental (E) and control (C) animals (Table 1).

Both experimental and control animals of this series were exposed to a single ionizing radiation. Experimental rats of the 1st series received xenogenic CSF once 24 hours after irradiation at a dose of 10 *mL/kg* body weight by intramuscular injection, whereas experimental rats of the 2nd series – repeated doses of 2 *mL/kg* body weight once in 3 days. Control rats of these series were injected with saline with the same frequency and dose.

Animals of the 1st series of the experiment were sacrificed by decapitation under thiopental anesthesia on the 3rd, 7th, 14th and 30th days after irradiation and animals of the 2nd series of the experiment – on the 7th, 14th and 30th days. Rats of all series (both experimental and control) were kept under standard vivarium conditions.

Bioethical considerations. All measurements and studies were carried out using measuring instruments that passed metrological calibration and additional equipment that was certified on the basis of the morphology department of the Central Research Laboratory of the Medical Academy named after S. I. Georgievsky. Study compliance with the basic bioethical norms is confirmed by the ethics committee of V. I. Vernadsky Crimean Federal University.

Study methods. Following isolation of thymus and carrying out the necessary macroscopic and organometry studies, the organ was fixed in a 10% solution of buffered neutral formalin, passed through a battery of alcohols of increasing concentration and embedded in paraffin. Serial histological sections 4 to 5 *μm* thick were stained with standard hematoxylin and eosin histological staining. Immunohistochemical staining was performed on sections 3 to 4 *μm* thick in accordance with the protocols of TermoScientific (USA) using the Quanto and DAB Chromogen imaging system. A positive reaction was assessed by the yellow-brown staining of the nucleus – for Ki-67 (clone sp6, TermoScientific) or cytoplasm – for CD68 (clone ab-1, TermoScientific) marker. The quantitative expression level of studied markers was expressed as a percentage of the immunopositive nuclei / cytoplasm (Ki-67 and CD68, respectively) area and the area of immunonegative staining (I_{ki67} and I_{CD68} indices, respectively).

Thymus histological slides were viewed and captured using an Olympus CX-41 cytomorphometric complex with Plan 4x ∞ / -, Plan 10x x / 0.25, Plan 40x x / 0.65, ∞ / 0.17 lenses. Using ImageJ software [*Schneider C et al., 2012*], the areas of the main structural and functional structures of the thymus (capsule and trabeculae, cortex, medulla) were measured. The results were expressed as values of relative area as a percentage of the total area of histological section. As an additional histomorphometric parameter, the cortico-medullary index (CMI) was determined. The cellular composition and the ratio of the major cell popula-

TABLE 1

Scheme of the experiment

Series	Experimental model	Terms of the experiment	Number of animals	
			E	C
S1	Single irradiation + single injection of CSF / saline at a dose of 10 *mL/kg*	3rd, 7th, 14th, 30th days	24	24
S2	Single irradiation + repeated injections of CSF / saline at a dose of 2 *mL/kg* once per 3 days	7th, 14th, 30th days	18	18

tions of various structural-functional zones of thymus (subcapsular zone, inner zone of the cortex, medulla) were evaluated on semithin sections with an immersion magnification.

Statistical analysis. Statistical data were analyzed using MS Excel and Statistica 10.0 (StatSoft, USA). Data distribution was normal (according to the results of Kolmogorov-Smirnov test [*Corder G, Foreman D, 2014*]. Thus, parametric statistical methods were used such as arithmetic mean and standard error of mean (SEM) (M ± m). Values were compared using non-parametric U-criterion of Mann-Whitney at a significance level of $\alpha = 5\%$. For all statistical evaluation, a probability of value $p<0.05$ was considered significant.

Results

Structural transformations of thymus in control rats on the 3rd day after irradiation were most pronounced and manifested in intensive edema of connective tissue, and loss of the typical structural organization. At the same time, a decrease in the relative cortex area (by 15.14% in comparison with the non-irradiated control, $p<0.05$) and an increase in the relative area of stromal-vascular component (by 89.64% in comparison with the non-irradiated control, $p<0.05$) was observed. All structural and functional areas of the thymus were characterized by a significant reduction in the density of cell population (in the inner zone of the cortex by 78.80% compared to non-irradiated control, $p<0.05$) due to decrease in the number of lymphoid cells (Fig. 1B).

Subpopulation of medulla thymocytes despite a decrease in their relative number was characterized by a shift towards larger cellular forms, some of which were in the stage of mitotic cycle. The activation of proliferative potential in the subcapsular zone of the cortex and in the thymic medulla was also confirmed by immunohistochemical analysis of Ki-67 marker (Fig. 2A). The pronounced destructive processes were accompanied by a statistically significant increase of immunohistochemical index of the macrophage CD68 marker compared with the non-irradiated control – in subcapsular zone by 242.11% (Fig. 2B), and in cortico-medullary zone – by 56.94% (both, $p<0.05$). Thus, starting from the 3rd day after irradiation, reparative processes begin to be activated on the background of a significant severity of thymus lesions.

Histological and cytological structure of thymus on the 7th, 14th and 30th days after irradiation are characterized by a gradual recovery, which was shown by an increase in density of cell population, mainly due to lymphoid cells, high proliferative potential of subcapsular zone of the cortex, as well as the medulla (Fig. 3).

Additionally, pronounced destructive and dystrophic changes in lymphoid and microenvironment cells, often becoming irreversible, as well as lesion of microvasculature and surrounding loose fibrous connective tissue was noted. The outcome of such transformations to the 30th day after irradiation was only partial restoration of thymic structure and its cell population along with the pronounced sclerotic changes in perivascular spaces and partially persisted histo- and cytopathological abnormalities.

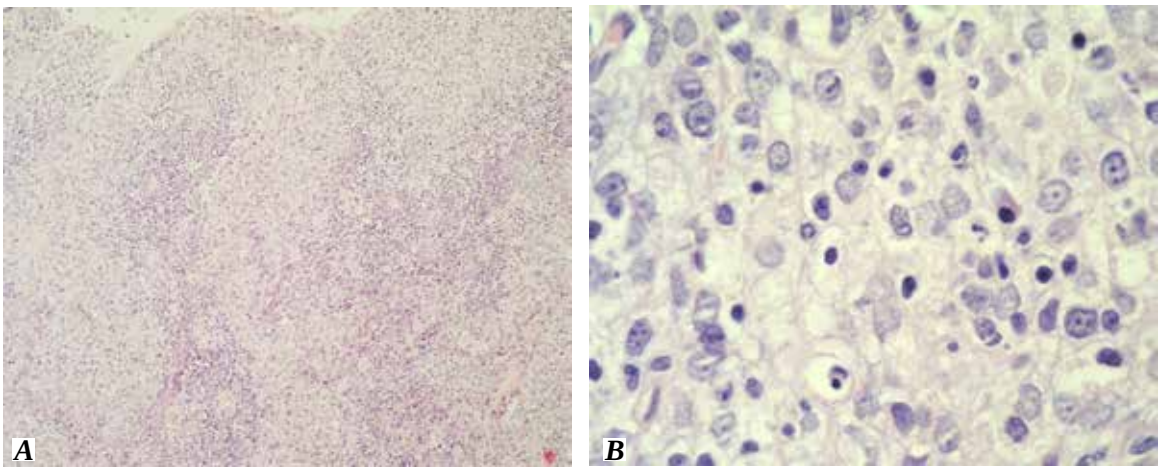

***Figure 1**. Cortex of the thymus of the control rat, 3rd day of the experiment.*
***A** – Inversion of the cortex and medulla of the thymus, decreasing of the cortex. H&E. ×100*
***B** – Severe depletion of the cell population of thymic cortex with multiple cells with signs of destruction. H&E. ×1000*

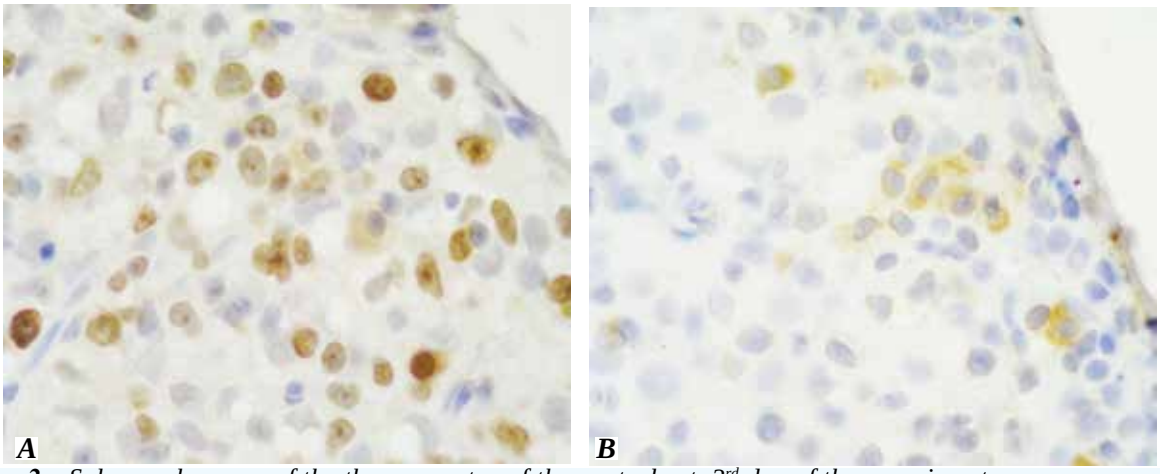

***FIGURE 2** – Subcapsular zone of the thymus cortex of the control rat, 3rd day of the experiment.*
***A** – Significant expression of the proliferation Ki-67 marker. ×1000*
***B** – Moderate expression of pan-macrophageal CD68 marker and nest-like accumulations of CD68+ cells. ×1000*

TABLE 2

Thymus histomorphometric parameters of irradiated control rats (M ± m)

	1st series of experiments (S1)				2nd series of experiments (S2)		
Period (days)	3	7	14	30	7	14	30
Cortex (%)	49.04±1.49	46.43±1.26	38.55±2.65	55.75±1.26	45.32±1.50	36.12±1.92	53.01±2.20
Medulla (%)	24.36±1.07	22.57±0.92	34.10±1.39	25.18±0.82	25.49±0.88	37.01±1.63	26.94±1.12
Capsule and inter-lobular septum (%)	26.59±0.56	31.00±0.73	27.35±1.80	19.99±0.28	29.19±1.01	26.87±0.87	20.05±0.69
Cortico-medullary index	2.05±0.15	2.12±0.15	1.16±0.12	2.26±0.12	1,78±0.18	0.98±0.10	1.97±0.16

So, on the 30th day of the experiment an increase in relative area of stromal-vascular components of thymus by 42.58% ($p<0.05$) and a decrease in relative number of lymphoid cells in inner cortex (by 18, 57%, $p<0.05$) with multiple cells with signs of destruction compared with the non-irradiated control was observed.

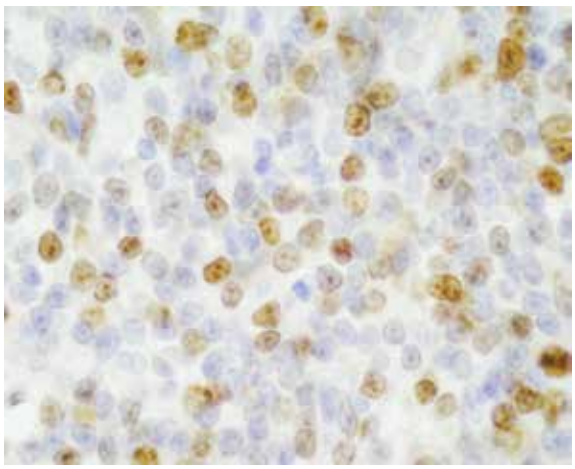

***FIGURE 3**. Medulla of the thymus of the control rat, 7th day of the experiment. Pronounced nuclear expression of the proliferative marker Ki-67. x1000*

Summary of histomorphometric data and quantitative determination of the expression level of Ki-67 and CD68 immunohistochemistry markers based on the analysis of control rats is presented in tables 2 and 3, respectively.

Analysis of the experimental rats of 1st and 2nd series of experiments with single or multiple administrations of xenogenic CSF during the post-radiation period has revealed a number of deviations from both the non-irradiated and irradiated control animals. On the 3rd day after irradiation the thymus of experimental rats was characterized by the same pronounced signs of radiation damage. Such transformations compared to non-irradiated control included inversion of the layers, decrease of the cortex area (by 25.29%, $p<0.05$) and corresponding increase of the medulla relative area (by 11.67%, $p<0.05$), as well as the pronounced reaction of microvasculature (Fig. 4).

Table 3

The expression level of Ki-67 (I_{ki67}) and CD68 (I_{CD68}) in various structural-functional zones of the thymus of irradiated control rats (M ± m)

Parameters	1st series of experiments (S1)				2nd series of experiments (S2)		
Period (days)	3	7	14	30	7	14	30
Immunohistochemistry Ki67 index (I_{ki67})							
Subcapsular zone	62.69±3.40	48.09±2.47	32.94±2.03	58.08±4.63	50.43±2.06	35.50±2.39	56.12±3.70
Inner zone of the cortex	37.52±3.24	32.62±2.22	35.50±1.37	39.38±2.39	30.43±1.98	37.97±2.11	41.84±2.57
Medulla	25.59±2.39	18.86±0.76	18.67±1.22	21.03±1.01	20.15±1.86	19.13±1.55	23.40±2.00
Immunohistochemical CD68 index (I_{CD68})							
Subcapsular zone	35.41±1.80	8.79±0.80	10.43±1.38	9.15±0.76	10.20±1.07	11.13±1.22	8.56±0.93
Cortico-medullary zone	22.23±2.98	10.24±2.98	12.25±1.19	11.03±0.89	9.45±1.60	13.00±1.61	12.34±1.57

Recovery of the thymus structure on the 7th, 14th and 30th days after irradiation in experimental rats was characterized by the same features as for irradiated rats without correction. Repopulation of the thymus was associated with the visual increase of the cortex, decreasing of connective tissue stroma and perivascular spaces edema, presence of a large number of blast forms and mitotically active cells in the thymus subcapsular zone, inner cortex and medulla (Fig. 5).

The similar transformations aimed at restoring histological and cytological structure of the organ in 2nd series of experiments (repeated parenteral administration of CSF) were more pronounced. These observations were confirmed by the histomorphometric data analysis (Table 4).

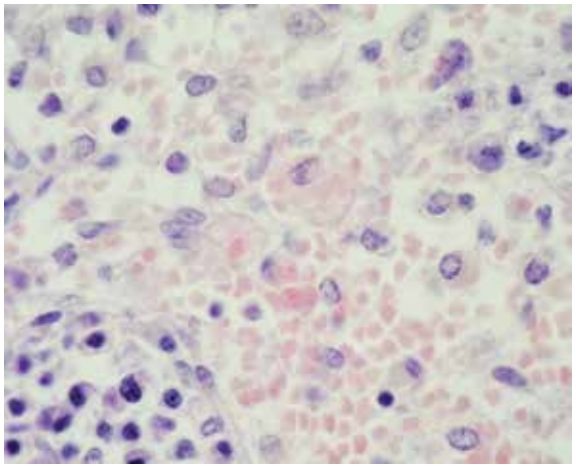

***Figure 4**. Thymus cortex of the experimental rats, 3rd day of the experiment. Irradiation + single administration of CSF at a dose of 10 mL/kg. H&E. ×1000.*

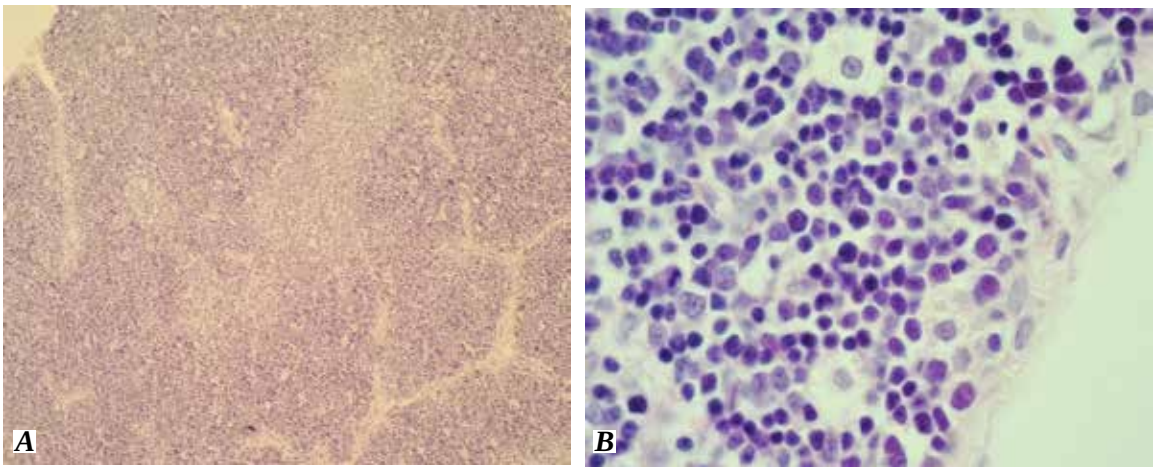

***Figure 5** . Thymus of experimental rats, 7th day of the experiment. Irradiation + repeated administration of CSF at a dose of 2 mL/kg.*
***A** – Recovery of both cortex and medulla structure, and the lobular structure of the organ. H&E. ×100.*
***B** – Repopulation of subcapsular zone with increasing number of active macrophages and lymphoblasts. H&E. ×1000.*

Repeated administration of CSF in low doses (2 *mL/kg*) on the 7th, 14th and 30th days after irradiation demonstrated substantial and statistically significant deviation from the respective parameters of thymus structural arrangement recovery in control irradiated animals. In most cases, these deviations from the control were more pronounced compared to the 1st series of experiments.

Attention is drawn to a significant increase in CD68+ cells in the thymus after administration of xenogenic CSF, and this phenomenon was most pronounced on the 7th and 14th days after irradiation, slightly decreasing by the 30th day of the experiment along with decrease in destructive processes caused by direct damage of ionizing radiation. Following a single high dose of CSF, I_{CD68} in the thymus cortico-medullary zone compared to irradiated control was increased (by 21.64% on the 7th day of the experiment [$p<0.05$] and by 11.90% on the 14th day of the experiment [$p>0.05$]). Morphologically, accumulations of macrophages in perivascular regions of cortico-medullary zone were found (Fig. 6).

On the contrary, repeated administration of CSF resulted in decrease in I_{CD68} of cortico-medullary thymic zone (by 45.50% on the 7th day of the experiment [$p<0.05$] and by 13.61% on the 14th day of the experiment [$p<0.05$]) along with a statistically significant increase in this parameter in the subcapsular zone of the cortex (by 81.88% on 7th day, by 40.61% on 14th day and by 15.85% on 30th day [compared to irradiated control; $p<0.05$]).

Summary of immunohistochemical Ki-67 and CD68 markers expression in the thymus of experimental rats in 1st and 2nd series of the experiments is presented in Table 5.

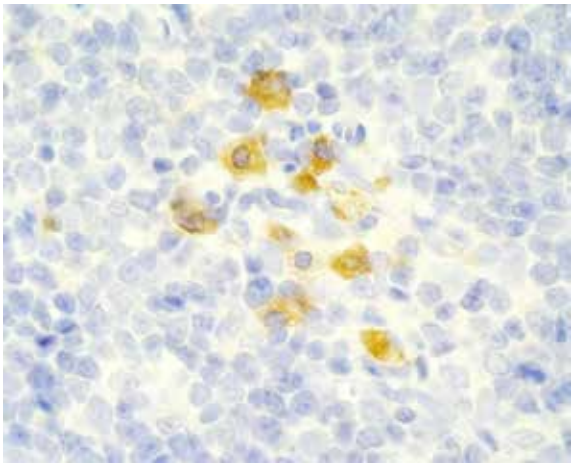

***FIGURE 6** – Thymus cortico-medullary zone of the experimental rats, 7th day of the experiment. Irradiation + single administration of CSF at a dose of 10 mL/kg. Perivascular accumulation and active migration of CD68+ cells is observed. CD68. x1000.*

DISCUSSION

The thymus is one of the first organs in organism that reacts to negative endogenous and exogenous factors among which ionizing radiation is not the least. High radiation sensitivity of thymocytes (mainly in cortex) [*Jinbo L, 2012*] and lability of cell population due to intrathymic migration and migration of progenitor cells from bone marrow [*Mitin A et al., 2012*] should be noted as a background of pronounced changes in thymic histological and cytological structural arrangement under the exposure to ionizing radiation. On the other hand, taking into account the imminent activation of hypothalamic-pituitary-adrenal regulation axis as a body's stress response along with the direct damaging effects of ionizing radiation on thymocytes, the glucocorticoid-mediated activation of apoptosis should also be considered [*Elmore S,*

TABLE 4

Thymus histomorphometric parameters of irradiated experimental rats in 1st and 2nd series of experiments (M ± m)

Parameters	1st series of experiments (S1)				2nd series of experiments (S2)		
Period (days)	3	7	14	30	7	14	30
Cortex (%)	43.01±2.20	46.34±3.94	49.70±1.47	58.84±1.36	62.09±1.27	58.14±1.33	59.99±0.92
Medulla (%)	30.71±1.95	23.08±2.61	28.64±1.11	23.74±0.78	19.54±1.57	25.61±1.31	21.66±0.48
Capsule and interlobular septum (%)	26.28±1.40	30.58±2.01	21.66±1.12	17.42±0.89	18.37±1.53	16.25±0.54	18.35±0.76
Cortico-medullary index	1.48±0.19	2.36±0.44	1.78±0.11	2.53±0.13	3.29±0.28	2.31±0.17	2.78±0.09

TABLE 5

The expression level of Ki67 (I_{ki67}) and CD68 (I_{CD68}) in various structural-functional zones of the thymus of the experimental rats (M ± m)

Parameters	1st series of experiments (S1)				2nd series of experiments (S2)		
Period (days)	3	7	14	30	7	14	30
Immunohistochemistry Ki67 index (I_{ki67})							
Subcapsular zone	51.41±2.78	44.67±2.38	43.95±1.92	28.88±2.11	73.09±2.52	33.78±1.77	57.06±1.50
Inner zone of the cortex	44.23±1.50	38.74±1.82	38.24±1.74	38.86±1.80	56.01±1.92	38.18±1.19	45,15±1,20
Medulla	21.55±1.26	22.98±0.94	23.96±1.71	23.40±1.02	40.95±1.64	20.73±0.72	28.27±1.34
Immunohistochemical CD68 index (I_{CD68})							
Subcapsular zone	26.66±2.22	9.26±0.97	11.27±1.41	9.15±1.46	15.99±0.73	14.67±0.80	10.60±1.10
Cortico-medullary zone	19.61±2.10	12.46±1.19	13.71±1.67	11.07±1.34	12.12±1.13	10.58±1.08	11.13±1.10

2006]. The identified time-related structural transformations of thymus correlated with changes in peripheral immune organs (mesenteric lymph nodes) under the same experimental conditions [*Kriventsov M, Kutsaya V, 2014*], and also with the data of some authors describing the post-radiation regeneration of thymus with two-wave kinetics [*Mitin A et al., 2012*].

Starting from the 7th day after irradiation, the immunohistochemistry study has revealed dynamics of proliferation (Ki67) and macrophageal (CD68) markers, indicating on restoration of thymic cell population, lost following exposure to ionizing radiation. This may happen due to migration of immune cell precursors from the bone marrow, monocyte recruitment, as well as due to activation of intrathymic lymphocyte proliferation, mainly in subcapsular zone.

Summarizing the data of histological, histomorphometric and immunomorphological analyses, the recovery process of the thymus in postradiation period without correction is characterized by two-wave kinetics with recession on the 3rd and 14th days and relative restoration on the 7th and 30th days after irradiation. Generally, it is consistent with the literature data, indicating that the period of primary lesion of thymus in a sublethal radiation injury accounts for 1 to 3 days, and the period of secondary lesions – 14 to 25 days after exposure [*Yarilin A, 1997*]. Such a wide range of secondary recession of thymus regeneration, presumably, is determined by the experimental models using different laboratory animals (rats, mice, guinea pigs), types and doses of ionizing radiation [*Butomo N et al., 2004*]. Whatever, thymus histological and cytological organization on the 14th day of observation was characterized by more pronounced involution compared with the previous and subsequent experimental periods. According to classical conception, such recession at a relatively remote period following a single dose total body irradiation is associated with the depletion of intrathymic reserves on the background of unresolved hematopoietic function and progenitor T-cell deficiency in bone marrow [*Gridley D et al., 2002*].

Evaluating the results obtained in a series of experiments with the use of xenogenic CSF as a radioprotective agent, it should be noted that parenteral administration of CSF has a pronounced effect on structural transformation of thymus in postradiation period. The revealed changes were generally linear and were focused on restoration of cytological and histological structure of thymus, largely depending on the dose and frequency of CSF administration. It can be concluded that a single injection of CSF at a dose of 10 mL/kg has excessive and short effect, resulting in aggravation of reactive changes in microvasculature during the early postradiation period followed by the peak of the restoration processes on the 14th day of the experiment.

Given the multidimensional relationship between nervous, endocrine and immune systems [*Barnard A et al., 2008*], the effects of paren-

teral administration of CSF may be caused both by direct exposure of biologically active substances on different organs and target cells [*Poon A et al., 1994*], and by indirect effect on hypothalamic-pituitary system, thyroid gland, adrenal glands or gonads. Most probably, various brain neurotransmitters and peptides [*Souza-Moreira L et al, 2011*], melatonin [*Malpaux B et al., 2002*], hypothalamus/neurohypophysis hormones [*Skinner D et al., 1997*] and cytokines [*Turnbull A, Rivier C, 1999*] should be considered as active substances in CSF.

On the other hand, the experimental data can be interpreted in terms of regulatory function of CSF in organism. Considering the whole organism and CSF as an integral humoral central nervous system environment, it is possible to suggest that a part of the regulatory neurohumoral influences is realized by transmitting a signal into the interstitial fluid, and then – into CSF, with its subsequent outflow along the circulation paths within the CNS as well as beyond its borders, as well as the outflow of the fluid into the venous and/or lymphatic circulation [*Johanson C et al., 2008*].

CONCLUSION

Structural changes of the thymus after a single exposure to ionizing radiation without correction are characterized by pronounced destructive changes and on the 3rd day of the experiment are presented by inversion of thymic layers, severe microvasculature reaction and interstitial space edema with a significant reduction of cell population with controversial changes in proliferative and macrophage activity. Histological organization of the thymus on 7th, 14th and 30th days after irradiation is characterized by persistence of destructive changes with partial recovery of histological and cytological structural arrangements of the organ.

A single parenteral administration of xenogenic cerebrospinal fluid has a pronounced, but short-term depressing effect, reaching its maximum on the 3rd day of the experiment and manifested by a decrease in cortico-medullary index and reducing cell population in the inner zone of thymic cortex. Repeated administrations of CSF at a dose of 2 mL/kg shows a significant stimulating effect on irradiated thymus at all time points of observation with a significant increase in proliferative activity of thymocytes and increasing the relative amount of CD68+ cells.

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