GENERAL AND SPECIFIC STRUCTURAL CHANGES OF LYMPHOID ORGANS IN THE HIGH DOSE APPLICATION OF SODIUM SELENITE

I. Putalova¹, O. Zayko², A. Sindireva³, O. Grinenko¹, I. Kosheleva¹

¹Department of Human Anatomy, Omsk State Medical University (OSMU), Omsk, Russia
²Peoples’ Friendship University of Russia, Moscow, Russia
³Department of Geoecology and Environmental Management, Tyumen State University, Tyumen, Russia

ABSTRACT
PURPOSE: to evaluate experimentally the features of structural and functional changes in lymphoid organs under the conditions of oral administration of high doses of sodium selenite and after its termination.

MATERIAL AND METHODS. The study was conducted on 30 sexually mature male Wistar rats, weighing 200-220 g. The morphometric research of the thymus, group lymphoid nodules, mesenteric lymph nodes, spleen, and laboratory investigation of blood were performed. The animals were divided into 3 groups depending on the nature of the study. The first (control) group consisted of intact rats. Animals of groups 2 and 3 were orally administered sodium selenite, at a rate of 5 mg/kg of mass for 5 days. Morphofunctional studies of lymphoid organs in group 2 were performed on day 6, in group 3 – on day 14.

RESULTS. The degree of severity and directional effect of structural and changes are determined by the functional specialization of the studied organs.

CONCLUSION. Signs of endotoxicosis indicate evident structural and functional changes in the lymphoid organs after five times administration of sodium selenite. Canceling the administration helps to improve the organism’s reserve capacity and adapt to altered metabolic processes.

Key words: thymus, aggregated lymphoid nodules, mesenteric nodes, spleen.

Selenium is a vital trace element for a human organism. It is a part of more than 30 proteins. A decrease in the content of this trace element in tissues can provoke a reaction of formation of toxic oxygen metabolites that damage cell membranes, which is the basis of morphofunctional changes in internal hormones; therefore, the leading mechanism of its biological action is antioxidanted. Cardiomyopathies, bone disorders, muscle and nerve tissue development, hypothyroidism, and cancer are classified as diseases associated with selenium deficiency. The soils of some territories in the Russian Federation contain little selenium. This implies a lack of selenium in organism of people living in these areas. At the same time, selenium compounds have a low therapeutic threshold, so a relatively small excess of their intake can cause poisoning. In these conditions, a significant load, for example, with uncontrolled use of selenium-containing drugs, falls on the lymphatic system and lymphoid organs which are responsible for detoxification and the homeostasis of the internal environment in the organism. These organs provide adaptation and compensation processes under various adverse impacts.

THE MAIN PURPOSE of our study is to evaluate experimentally the features of structural and functional changes in lymphoid organs under the conditions of oral administration of high doses of sodium selenite and after its termination.

MATERIAL AND METHODS
The experimental study was conducted on 30 sexually mature male Wistar rats, weighing...
200-220 g. The objects of the study were the thymus, group lymphoid nodules of the ileum (Payer's patches), mesenteric lymph nodes, spleen, and blood. The animals were divided into 3 groups depending on the nature of the study. The first (control) group consisted of 10 intact rats. Animals of groups 2 and 3 were orally administered a toxic dose of sodium selenite, at a rate of 5 mg/kg of mass for 5 days (20 individuals). All manipulations with animals were approved by the Local Ethical Committee of OSMU and performed in accordance with the "Rules for Working with Experimental Animals" (Annex to Order No. 755 of 12.08.1977) and the provisions of the Helsinki Declaration of 2000, 2002, 2004, 2008, 2013. Morphofunctional studies of lymphoid organs in the group 2 were performed on day 6 (after five times administration of sodium selenite), in the group 3 – on day 14 (on day 9 after drug withdrawal). The material was studied using classical morphological methods and a set of functional methods (registration of the content of low and medium molecular weight substances). Morphometric analysis of the structural components of lymphoid organs was performed using microscopes MBS-10 and LOMO Mikmed-2, taking into account modern approaches adopted when performing morphological studies. Registration of the content of low- and medium-weight substances was performed using the method of M. Ya. Malakhov (1995) on the LOMO SF – 26 spectrophotometer in the zone of wavelengths from 238 nm to 298 nm with an interval of 4 nm. Statistical analysis of quantitative data was carried out in accordance with the principles of evidence-based medicine, using variational analysis, applying the method used for the normal distribution of indicators. The differences were considered significant at p<0.05.

RESULTS, CONCLUSION

According to our data, the total area of the mesenteric lymph node (MLN) in the control group is 12.88+0.77 mm². The structure of the node is dominated by the area of the medulla, which allows it to be classified as a fragmented type (Figure 1). For nodes of this morphtype, the function of lymph transport is more characteristic. The share of the capsule is 3.5% in the MLN structure. Throughout, it has a different thickness. In parts it is thin, in parts, on the contrary, it is thickened, due to the end sections of afferent lymphatic vessels. Afferent lymphatic vessels open into the subcapsular sinus above the interstitial part of the cortical substance. It is believed that this area of the interstitial zone determines the nature of further migration of cells that entered the node with afferent lymph. The area of subcapsular space in animals of the control group is relatively large for this experiment (0.40+0.058 mm2). This confirms our conclusion that mesenteric lymph nodes are normally characterized by active drainage function, which is due to their localization.

![Figure 1. Fragmented mesenteric lymph node morphotype of intact rats. Enlarged afferent lymph vessels. Hematoxylin and eosin staining. Digital magnification.](image)

6 days after five times of administration of high dose of sodium selenite in animals blood, a decrease (by 21.3%) in the number of red blood cells and the concentration of hemoglobin (by 25.7%) was determined, which leads to hypoxia. The consequence of
this is an increase in the intensity of anaerobic glycolysis, which is expressed in an increase in the concentration of lactic acid in rat’s blood (by 46.4%). These processes are associated with increased production of active oxygen metabolites.

During this period, the thymus structure significantly reduces the values of indicators (by 2 times or more) of the total cross-section area, areas of cortex and medulla of thymus, of capsules and cortical septums (by 1.5 times). The number of lymphoid cells decreased in all structural and functional zones. These changes correspond to signs of accidental thymus involution. A moderate negative correlation between the thymus and secondary lymphoid organs may indicate the migration of mature lymphocytes.

The total area of the MLN is reduced by 17 %. In the structure of the node, the area of the cortical substance increases by 63% and its share increases by almost 2 times. The morphotype of the node changes to compact, which is indicated by the cortex/medulla (K/M) index (in the control 0,60±0,04; 3,01±0,46 – in the 2nd group of animals). For nodes of this morphotype, the function of lymph detoxification is characteristic (Figure 2).

In the structure of aggregated lymphoid nodules of the ileum, a decrease in the number and area of primary lymphoid nodules and an increase in the number and proportion of secondary lymphoid nodules were determined (Table 1). As a part of the latter, the area of germinal centers increases (by 57%). However, the total number of cells in the germinal centers decreases and degenerative (destroyed) cells increases.

Table 1. Areas of structural components of rat Payer’s patches are normal, with oral administration of a toxic dose of sodium selenite and after its cancellation (M ± m)

| Investigated parameters | the control | on day 6 | on day 14 |
|-------------------------|-------------|----------|----------|
| Primary lymphoid nodule | R           | 3,25±0,28 | 2,60±0,20 * | 1,40±0,12*N |
|                         | mm²         | 0,78±0,07 | 0,25±0,02 * | 0,14±0,01*N |
|                         | %           | 11,32±1,06| 4,14±0,41 * | 4,13±0,41 *  |
| Secondary lymphoid nodule | R           | 4,25±0,42 | 7,40±0,70 | 6,20±0,43 * |
|                         | mm²         | 3,52±0,32 | 4,06±0,16 | 1,96±0,10*N |
|                         | %           | 44,56±0,43| 60,37±1,51 * | 57,29±1,75 * |
| Germinal centre           | mm²         | 1,06±0,10 | 1,96±0,17 * | 0,71±0,05*N |
|                         | %           | 15,46±1,44| 29,06±2,85 * | 21,12±1,77 |
| Mantle zone              | mm²         | 1,68±0,16 | 2,10±0,02 | 1,25±0,12 N |
|                         | %           | 24,11±2,47| 35,08±1,52 | 37,26±3,72 |
| Internodular zone         | mm²         | 2,90±0,29 | 1,96±0,19 * | 1,30±0,06 *  |
|                         | %           | 42,19±0,29| 34,39±1,42 * | 38,58±0,88N |
| Total area               | mm²         | 7,20±0,69 | 6,27±0,54 | 4,66±0,14*N |

Note: R is the number
* - the difference is significant when compared with similar control indicators (p<0,05),
N – difference is significant when compared with similar indicators of the So6 group (p<0,05)

In the spleen, the changes affected only the white pulp. The marginal zone increased the area of secondary lymphoid nodules by 20% (Figure 3).
After five times administration of sodium selenite, structural and functional changes in the lymphoid organs are aimed at enhancing drainage and detoxification functions. However, it doesn’t respond to the organism’s needs. In blood, the content of substances of catabolic origin exceeds the control values by 2 times, and the values of the leukocyte index of intoxication and the number of neutrophilic granulocytes are maximally reduced.

9 days after the withdrawal of the introduction of sodium selenite (on day 14), the thymus structure is not restored, the values of the areas of the main structural zones remain less than the control ones. On the contrary, the number of lymphoid and epithelial cells is still reduced. The number of degenerating cells and macrophages increases. In parallel, we determined the tendency to increase the area of the cortical substance and increase the number of medium-sized lymphocytes, which does not exclude the possibility of thymus regeneration. During this period, the most severe structural transformations were found in the spleen. The total area of the cut was reduced by 1.5 times, due to both red and white flesh. The maximum changes were found in the periarteriolar lymphoid sheath, its area decreased by 2 times. In the mesenteric lymph node, positive changes in the main structural components were observed after the administration was canceled. Positive dynamics of the main structural components was observed in the mesenteric lymph node after the withdrawal of sodium selenite. In the cortical substance, the area of the internodular zone increased (by 2 times) to the control values, which may indirectly indicate the activation of the drainage function of the node. The increase in the number of cells in the state of mitosis, plasmocytes indicates the possibility of reparative processes. The total area of the aggregated lymphoid nodules continues to decrease both in comparison with the control and in comparison with the value on day 6. The number of secondary lymphoid nodules increases in comparison with the control, but the value of their area decreases and becomes smaller than the control ones (by 44%). After discontinuation of administration, the number of lymphocytes increases, and the value of the proportion of the internodular zone returns to normal.

Thus, after the withdrawal of sodium selenite, structural changes in the thymus, spleen, and group lymphoid nodules indicate that the lymphoid organs cannot fully provide homeostatic function in the organism. This is confirmed by maintaining a high level of low and medium molecular weight substances in blood. At the same time, the positive dynamics of changes in the mesenteric lymph node, thymus (increase in lymphoid cells), normalization of leukogram indicators suggest reserve capabilities of lymphoid organs, which the speed and quality of recovery processes depend on.

REFERENCES
1. Zayko O. A., Sindireva A.V., Putalova I. N., Konvay V. D., The effect of toxic doses of selenium on the processes of peroxidation in the blood and mesenteric lymph nodes of rats. Medical science and education of Ural, 2:57-60, 2009.
2. Sindireva A. V., Putalova I. N., Alexandrovskaya E. Yu., Zaiko, O. A., Konvay V. D., Influence of selenium contained in feed, on the structural and functional changes in animal organs. Vestnik OrelSAU, 5 (62): 94-99, 2016.