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AGE FEATURES OF THE THYMOCITE RNA RESPONSE IN THE THYMUS STRUCTURAL AND FUNCTIONAL AREAS TO BLOOD CIRCULATION DISORDERS IN THE POOL OF CAROTIC ARTERIES IN RATS

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Abstract

Introduction. Ischemic-reperfusion injuries of the brain are accompanied by immunological disorders, which in their turn can extend post-ischemic changes in the brain. The studies of structural and functional changes in the thymus cells caused byischemia-reperfusion of the brain can supplement the existing understanding of the mechanism of the above interaction.

The aim of the study. To investigate the reaction of thymocyte RNA in the structural and functional areas of the thymus gland to blood circulation disturbances in the carotid artery pool.

Results. In rats of both age groups, cerebral ischemia-reperfusion reduces the content of RNA in the thymus cells in all the structural and functional areas of the thymus except the deep cortical one in one-month rats and the sub-capsular one in three-month rats.

Conclusions. Acute circulatory disorders in the pool of the carotid arteries result in certain changes in RNA content in all the structural and functional areas of the thymus with age-related and structural features available.

Key words: incomplete global cerebral ischemia-reperfusion; cells of the thymus lymphoid population.

Nowadays, there is no doubt concerning the presence of neuroimmune interaction mediated by biologically active substances formed in the structures of the brain and thymus and participating in both neurochemical processes of the brain (by changing the conformation of various enzymes and synaptic proteins) as well as in regulatory mechanisms of the immune system[1-3]. Therefore, pathological conditions of the central nervous system become a cause of disturbances of the neuroimmune relationships leading to dysregulation of processes in the immune system. Cerebral ischemia, as a result of not only impaired cerebral circulation, but of many extreme conditions in medicine as well, is undoubtedly associated with immunological disorders [5, 6]. In their turn, they are able to aggravate the consequences of ischemia-reperfusion injury to the brain. Therefore, the studies of structural and functional changes in the thymus cells caused by cerebral ischemia-reperfusion are relevant in the context of searching the means to normalize neuroimmune relations under the above experimental conditions.

The aim of the study: to investigate the reaction of thymocyte RNA in the structural and functional areas of the thymus gland to blood circulation disturbances in the carotid artery pool.

Materials and methods of the study. 20-minute partial global cerebral ischemia followed by reperfusion was simulated in male albino laboratory rats of one and three months of age followed by reperfusion. The animals were removed from the experiment by decapitation under calypso anesthesia on the 12^{th} day after cerebral ischemia-reperfusion simulation. The thymus was placed in Buen's solution for 18 hours and after standard histological processing, it was embedded in paraffin. Several sections 5 μm thick were prepared. To detect RNA, the sections were deparaffinized, rehydrated in decreasing concentrations of ethanol, and stained with Einarson's gallocyanine-chromium alum. The histological sections were analyzed in the digital image analysis system VIDAS–386 (Kontron Electronik, Germany) under the fluorescent microscope AXIOSKOP (Zeiss, Germany). Total RNA content was determined in randomly selected sections of the thymus cortex and medulla. The microscope AXIOSKOP (Zeiss, Germany) and the digital image analysis system VIDAS 2.5 (Kontron Electronik, Germany) were used for mathematical classification analysis. The results were processed on an IBM-compatible personal computer using a package of the applied and statistical programs VIDAS 2.5 (Kontron Electronik,

Germany) and EXCELL MS Office 2000 (Microsoft Corp., USA) and Student's t-test.

Results and discussion. In the subcapsular zone of the thymus of one-month-old animals, ischemia caused a decrease in the content of RNA in unchanged lymphoblasts, large and medium thymocytes, as well as lymphoblasts and large lymphocytes with signs of destruction (table. 1). At the same time, the RNA content increased in small normal and destructive lymphocytes and those cells that underwent apoptosis.

Table 1 - RNA content (optical density units) in the cells of the lymphoid population of the sub-capsular area of the thymus in the control and cerebral ischemia groups of rats of different ages (M±m)

Observation group	Classes of the lymphoid population cells	Unchanged cells	Cells with destruction signs
1 month			
Control	Lymphoblasts	0,207±0,003	0,192±0,003
	Large lymphocytes	0,230±0,001	0,224±0,002
	Medium lymphocytes	0,252±0,001	0,230±0,003
	Small lymphocytes	0,254±0,001	0,243±0,002
	Cells with apoptosis signs	0,262±0,003	
Cerebral ischemia-reperfusion	Lymphoblasts	0,185±0,004*	0,172±0,004*
	Large lymphocytes	0,213±0,002*	0,195±0,003*
	Medium lymphocytes	0,247±0,002*	0,230±0,004
	Small lymphocytes	0,267±0,0008*	0,257±0,003*
	Cells with apoptosis signs	0,278±0,003*	
3 months			
Control	Lymphoblasts	0,167±0,002#	0,156±0,002#
	Large lymphocytes	0,191±0,001#	0,173±0,003#
	Medium lymphocytes	0,206±0,001#	0,188±0,003#
	Small lymphocytes	0,211±0,0005#	0,202±0,002#
	Cells with apoptosis signs	0,212±0,003	
Ischemia	Lymphoblasts	0,176±0,002*#	0,161±0,003#
	Large lymphocytes	0,210±0,002*	0,194±0,004*
	Medium lymphocytes	0,246±0,001*	0,215±0,004*#
	Small lymphocytes	0,262±0,001*#	0,232±0,004*#
	Cells with apoptosis signs	0,258±0,003*	

Note: here and in the following tables: probability of changes regarding indices: * – in control animals; # – reliability of age differences of the corresponding indices

In three-month rats, post-ischemic changes in the sub-capsular area of the thymus

consisted in an increase of RNA content in all the cells of the lymphoid population. The analysis of age characteristics demonstrated that in animals from the control group, RNA content in lymphocytes significantly dominated in one-month animals. However, ischemia significantly reduced these age-related differences due to an increase of RNA content in all the types of intact and destructive thymus cells.

In the deep cortex of the thymus of one-month rats, ischemia caused an increase in RNA content in all the lymphocytes, except unchanged and destructive lymphoblasts and destructive large lymphocytes (Table 2).

Table 2 - RNA content (optical density units) in the cells of the lymphoid population of the deep cortex of the thymus in the control and cerebral ischemia groups of rats of different ages (M±m)

Observation group	Classes of the lymphoid population cells	Unchanged cells	Cells with destruction signs
1 month			
Control	Lymphoblasts	0,224±0,003	0,220±0,0043
	Large lymphocytes	0,240±0,002	0,240±0,003
	Medium lymphocytes	0,259±0,001	0,254±0,004
	Small lymphocytes	0,275±0,001	0,267±0,003
	Cells with apoptosis signs	0,277±0,007	
Ischemia	Lymphoblasts	0,230±0,003	0,214±0,003
	Large lymphocytes	0,256±0,002*	0,241±0,003
	Medium lymphocytes	0,295±0,002*	0,296±0,003*
	Small lymphocytes	0,321±0,0007*	0,310±0,002*
	Cells with apoptosis signs	0,338±0,002*	
3 months			
Control	Lymphoblasts	0,210±0,004#	0,193±0,005#
	Large lymphocytes	0,216±0,002#	0,218±0,004#
	Medium lymphocytes	0,242±0,002#	0,243±0,006
	Small lymphocytes	0,265±0,001#	0,254±0,004#
	Cells with apoptosis signs	0,282±0,007	
Ischemia	Lymphoblasts	0,174±0,002*#	0,171±0,003*#
	Large lymphocytes	0,191±0,002*#	0,192±0,003*#
	Medium lymphocytes	0,230±0,002*#	0,231±0,004#
	Small lymphocytes	0,255±0,0006*#	0,226±0,004*#
	Cells with apoptosis signs	0,254±0,002*#	

RNA content in the thymus cells of three-month rats was significantly lower than that

of one-month animals except medium lymphocytes with the signs of destruction and apoptotic cells. Cerebral ischemia-reperfusion significantly reduced RNA content in all the lymphoid cells of the thymus area of three-month animals enhancing age differences.

Post-ischemic changes in RNA content in the intralobular perivascular spaces of the thymus except small unchanged and apoptotic lymphocytes with an increased indicator are shown in Table 3.

Table 3 - RNA content (optical density units) in the cells of the lymphoid population of the intralobular perivascular spaces of the thymus in the control and cerebral ischemia groups of rats of different ages (M±m)

Observation group	Classes of the lymphoid population cells	Unchanged cells	Cells with destruction signs
1 month			
Control	Lymphoblasts	0,188±0,003	0,190±0,004
	Large lymphocytes	0,211±0,002	0,208±0,003
	Medium lymphocytes	0,245±0,001	0,222±0,004
	Small lymphocytes	0,255±0,0007	0,245±0,003
	Cells with apoptosis signs	0,255±0,003	
Ischemia	Lymphoblasts	0,158±0,003*	0,143±0,004*
	Large lymphocytes	0,195±0,002*	0,186±0,004*
	Medium lymphocytes	0,240±0,001*	0,214±0,006
	Small lymphocytes	0,265±0,0008*	0,248±0,004
	Cells with apoptosis signs	0,285±0,004*	
3 months			
Control	Lymphoblasts	0,201±0,003#	0,203±0,005#
	Large lymphocytes	0,241±0,002#	0,225±0,005#
	Medium lymphocytes	0,280±0,001#	0,259±0,005#
	Small lymphocytes	0,290±0,001#	0,277±0,005#
	Cells with apoptosis signs	0,305±0,006#	
Ischemia	Lymphoblasts	0,120±0,002*#	0,116±0,003*#
	Large lymphocytes	0,140±0,002*#	0,131±0,004*#
	Medium lymphocytes	0,180±0,001*#	0,144±0,004*#
	Small lymphocytes	0,195±0,0007*#	0,165,±0,004*#
	Cells with apoptosis signs	0,190±0,004*#	

RNA content in this thymus area of three-month rats from the control group was higher in all the lymphoid cells than in one-month rats. However, due to cerebral ischemia it decreased more significantly in one-month rats, which caused reversal age differences.

In the cortical area of the thymus of one-month rats, RNA content in all the cells significantly decreased resulting from ischemic brain damage (Table 4). Age-related differences in RNA content in the cortical area of the thymus of animals from the control

group were insignificant. In three-month rats, the index was higher in large normal lymphocytes and lower in destructive small ones.

Table 4 - RNA content (optical density units) in the cells of the lymphoid population of the medullar area of the thymus in the control and cerebral ischemia in rats of different ages (M±m)

Observation group	Classes of the lymphoid population cells	Unchanged cells	Cells with destruction signs
1 month			
Control	Lymphoblasts	0,151±0,002	0,151±0,004
	Large lymphocytes	0,192±0,002	0,166±0,004
	Medium lymphocytes	0,229±0,001	0,175±0,005
	Small lymphocytes	0,233±0,0008	0,224±0,004
	Cells with apoptosis signs	0,236±0,004	
Ischemia	Lymphoblasts	0,127±0,002*	0,130±0,003*
	Large lymphocytes	0,160±0,002*	0,143±0,003*
	Medium lymphocytes	0,200±0,001*	0,146±0,004*
	Small lymphocytes	0,196±0,0008*	0,173±0,004*
	Cells with apoptosis signs	0,200±0,004*	
3 months			
Control	Lymphoblasts	0,156±0,003	0,154±0,005
	Large lymphocytes	0,199±0,001#	0,167±0,004
	Medium lymphocytes	0,230±0,001	0,189±0,006
	Small lymphocytes	0,231±0,0009	0,208±0,004#
	Cells with apoptosis signs	0,238±0,005	
Ischemia	Lymphoblasts	0,088±0,002*#	0,088±0,002*#
	Large lymphocytes	0,132±0,001*#	0,103±0,003*#
	Medium lymphocytes	0,166±0,0008*#	0,115±0,003*#
	Small lymphocytes	0,152±0,0008*#	0,133±0,003*#
	Cells with apoptosis signs	0,154±0,004*#	

Ischemia changed RNA content in all the thymus cells of three-month rats resulting in age-related differences in all the cell types.

The results obtained are indicative of the fact that incomplete global cerebral ischemia is associated with disregulatory changes in the morphological functional state of the thymus, which corresponds to the concept of functional integrity of the components of the neuro-immune-endocrine systems [10, 11, 12]. The structural features of the thymus response to

cerebral ischemia-reperfusion injury can be explained by the individuality of the functional purpose of each of these investigated areas in the hierarchy of relationships within the thymus [4, 7]. Varying degrees of dependence on the regulatory effects of the central nervous system and neuro-endocrine imbalance are accompanied by cerebral ischemia [1, 8, 9, 13].

Conclusions. Acute circulatory disorders in the pool of the carotid arteries result in certain changes in RNA content in all the structural and functional areas of the thymus with age-related and structural features available.

Author Contributions

The authors agree on equal distribution of partial participation.

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Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

All information is publicly available and data regarding this particular patient can be obtained upon request from corresponding senior author.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The authors declare that there are no conflicts of interest.

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