

of humoral immunity which may be a trigger for the development of autoimmune diseases and other inflammation-induced processes. Given the introduction of L and PL-308 a significant decrease of BSC was observed (respectively for L – 6.70 ± 0.80 %, PL-308 – 2.50 ± 0.50 %, $p \leq 0.05$). It should be noted that the compound PL-308 was more effective because it credibly reduced BSC even with respect to the comparator of L. The leptin level in the conditions of OVE and in the combination of OVE with MS tended to decrease compared to the intact group (respectively 8.20 ± 0.15 ng / ml and 8.10 ± 0.10 ng / ml against 9.00 ± 0.30 ng / ml, $0.05 < p < 0.1$) which may be due to the short duration of the experiment and, as we stated already, may be associated with the formation of insulin resistance at this stage [3]. The compounds L and PL-308 credibly increased the leptin level in comparison with OVE and OVE+MS (respectively L 11.30 ± 0.80 ng / ml, PL-308 – 10.00 ± 0.80 ng / ml, $p \leq 0.05$), herewith the PL-308 indicators were not significantly different from intact control. It should be noted that L has a thymomimetic, cholinergic and nicotinic effect which promotes the increase of insulin receptors on insulin-dependent cells to which adipocytes belong, and they affect the intensification of metabolic processes and the reduction of manifestations of the state of insulin resistance in conditions of estrogen deficiency. Thus, the reduction of cytotoxicity and the increase / normalization of leptin level under the influence of immunomodulatory compounds indicate the feasibility of immunocorrection application during menopause to reduce the manifestations of MS. Prospects for further scientific research. The obtained data indicate the prospect of further study of the compound PL-308 especially given the fact that acute toxicity estimation of the compound PL-308 is considered as class 5 (almost non-toxic compounds) while levamisole is considered as class 4 (low-toxic compounds).

References

1. Bairey Merz C.N., Ramineni T., Leong D. Sex-specific risk factors for cardiovascular disease in women-making cardiovascular disease real // *Curr. Opin. Cardiol.* – 2018. – V. 33, N. 5. – P. 500-505.
2. Chunhua Yin, Lumei Kang, Cong Lai et al. Effects of 17β -estradiol on leptin signaling in anterior pituitary of ovariectomized rats // *Exp. Anim.* – 2017. – V. 66, N. 2. – P. 159-166.
3. Kozar V. V., Yeriomenko R. F., Dolzhikova O. V. et al. Changing of Leptin concentration in ovariectomized rats with metabolic syndrome in the conditions of immune activity compounds administration // *Ukrainian Journal of Medicine, Biology and Sports.* – 2018. – V. 3, N 1(10). – P. 40-43.

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FEATURES OF DISTRIBUTION OF SOYBEAN AGGLUTININ (SBA) RECEPTORS IN THE EXTRACELLULAR MATRIX OF THE MENISCI OF RAT KNEE JOINT AFTER INTRAFETAL INJECTION OF ANTIGENS

Abrosimov Yu.Yu.

Zaporizhzhia State Medical University, Zaporizhzhia, Ukraine

Relevance. The results of previous investigations at the Department of Human Anatomy of Zaporizhzhia State Medical University have shown that intrafetal injection of antigens leads to changes in the rates of morphogenesis of fetal organs and tissues, that can be used for the modeling of the syndrome of undifferentiated connective tissue dysplasia in rats [3]. Lectins are informative molecular probes that can detect glycoconjugates in cells and tissues. Lectins and their receptors provide extracellular, cell-matrix interactions, participate in the regulation of proliferation, differentiation and apoptosis of cells [1, 2]. Previously we have shown that distribution of the receptors to other lectins (WGA, VSA) differs in intact knee joint menisci and after intrauterine antigenic effect [4, 5].

Aim of the work was to establish the features of distribution of Soybean agglutinin (SBA) receptors in the extracellular matrix of the menisci of rat knee joint after intrafetal injection of antigens.

Material and methods. Menisci of knee joints were studied in 160 white laboratory rats from the 1st to the 90th days of life. Group I – 60 intact rats. Group II – 60 experimental rats – the offspring of female rats, which on the 18th day of the dated pregnancy underwent the injection of purified staphylococcal toxoid (1:10, 0.05 ml) according to the method of professor N.A. Voloshyn (1981). 40 rats of group III after injection of saline solution served as control. When working with animals we were guided by «European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes» (Strasbourg, 18.03.86) and Law of Ukraine «On the protection of animals against cruel treatment» (№ 3447-IV). Receptors for Soybean agglutinin (SBA) in the histological sections were detected using standardized sets of SBA-HRP (RPC «Lectinotest»). The imaging was carried out in the diaminobenzidine-hydrogen peroxide system. The intensity of the deposition of benzidine label was assessed semi-quantitatively.

Results. On the 1st day after birth the intensity of the benzidine label deposition in the menisci of all groups of rats causes the light-brown staining (+) of extracellular matrix of the inner zone and yellow-brown of the outer one (++) . These revealed features of the contents of N-acetyl-D-galactosamine in extracellular matrix of menisci zones persist until the end of the first week of postnatal life. The visceral part of the joint capsule covering the menisci in all groups of animals is most intensely stained in brown (+++) color, which is not changed during three months of observation and does not depend on the antigen influence.

On the 11th day after the birth in the menisci of intact and control groups the level of SBA receptors increases in extracellular matrix of the inner zone (++) . However, in experimental rats, the amount of N-acetyl-D-galactosamine residues remains at the previous level (+). The staining intensity of the outer zone structures of the menisci of all groups of rats does not change and does not differ between groups (++) .

On the 14th day of postnatal life, the quantity of Soybean agglutinin receptors (SBA) in the extracellular matrix of the inner meniscus zone of all groups of animals is not changed. In experimental animals the intensity of the benzidine label deposition in the extracellular matrix of this zone is reduced. In the outer zone there is a decrease in the content of N-acetyl-D-galactosamine residues in intact and control animals (+). Instead, in antigen-treated animals level characteristic for the previous observation period (++) remains, which exceeds the intensity of the benzidine label deposition in the control groups. This level of N-acetyl-D-galactosamine residues remains until the end of the third week of life inclusively.

Further, on the 30th day after birth, the quantity of receptors to the Soybean agglutinine (SBA) in both zones of the menisci in rats of intact and control groups does not change. In experimental animals there is an increase in the content of N-acetyl-D-galactosamine residues in the inner zone with simultaneous decrease in the outer one to the level of intact animals.

On the 45th day of life in the inner zone of the menisci in rats of all research groups a decrease in the content of SBA receptors (+) was observed. The intensity of the benzidine label deposition in the outer zone remains unchanged (+). Such affinity for Soybean agglutinin receptors persists until the end of the 90th day of observation period in all animal groups. Since the 45th day, there is an increased deposition of benzidine label on the cell membrane of large-sized, vacuolated fibrochondrocytes located in the deep regions of the cartilage tissue. Subsequently, there is cartilage resorption and subchondral bone formation in their place.

Thus, the content of Soybean agglutinin receptors (SBA) in the intact and control group increases in the inner zone (from + to ++) on the 11th-30th days of life and decreases

on the 45th. In the outer zone there is a decrease in the quantity of N-acetyl-D-galactosamine residues (from ++ to +) on the 14th day. Intrauterine injection of antigens leads to changes in the rate of distribution of Soybean agglutinin (SBA) receptors in both menisci from the 11th to the 30th day of life.

References.

1. Antoniuk V. O. (2005). *Lektyny ta yikh syrovynni dzherela [Lectins and their raw materials sources]*. Lviv: Kwart. [in Ukrainian].
2. Voloshyn N. A., Hryhorieva E. A. (2005). *Lektyny zhyvotnogo i rastitel'nogo proiskhozhdeniya: rol' v protsessah morfogeneza [Lectins of animal and vegetable origin: role in the processes of morphogenesis]*. Zhurnal AMN Ukrainy. 11, 2, 223–237. [in Russian].
3. Voloshyn N. A., Hryhorieva E. A. (2009). *Ekspyrymental'naya model' razvitiya sindroma nedifferentsirovannoy displazii soedinitel'noy tkani [Experimental model of the development of undifferentiated connective tissue dysplasia syndrome]*. Patologiya. 6, 1, 39–42. [in Russian].
4. Abrosimov Yu. Yu. (2018). *Features of distribution of Vicia sativa agglutinin (VSA) receptors in the extracellular matrix of the menisci of rat knee joint after intrafetal injection of antigens*. Biological Markers in Fundamental and Clinical Medicine (collection of Abstracts), 2(2), 68-69. <https://doi.org/10.29256/v.02.02.2018.escbm59>
5. Abrosimov Yu. Yu. (2018). *Features of distribution of receptors to the wheat germ agglutinin (WGA) in the extracellular matrix of the menisci of rat knee joint after intrafetal injection of antigens*. Biological Markers in Fundamental and Clinical Medicine (collection of Abstracts), 2(1), 60-62. <https://doi.org/10.29256/v.02.01.2018.escbm26>

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POSSIBILITIES OF GLYCOGEN SYNTHASE KINASE-3B LEVEL USE AS A BIOMARKER OF THE COMBINED PRECONDITIONING IN THE ISCHEMIC BRAIN

Levchenkova O.S., Korneva Yu.S., Novikov V.E., Dorosevich A.E.
Smolensk State Medical University, Russia

One of the effective methods to increase an organism resistance to cerebral ischemia is preconditioning (PreC) [1,2]. Obviously, the role of the glycogen synthase kinase-3 β (GSK-3 β) pathway is to regulate mitochondrial permeability transition pore (mPTP), a principle trigger of apoptosis and even necrosis [3,4]. The aim of this study was to analyze whether the combined PreC influences the brain GSK-3 β level in cerebral ischemia; and compare the data with morphological changes in the most vulnerable to hypoxia CA1 and CA3 hippocampus fields.

Materials and Methods. All experiments were conducted with Wistar rats. The protocol of our investigated PreC method consisted of the pharmacological agent application (3,5-diamino-1,2,4-thiadiazole – amtizol in dose 25 mg/kg, intraperitoneally) on the 1st, 3rd and 5th day of the experiment. On the 2nd, 4th, 6th day the rats were subjected to the moderate hypobaric hypoxia (410 mm Hg, 60 min) [2], followed by cerebral ischemia through bilateral ligation of the common carotid artery. The ischemia was performed under 8% solution of chloral hydrate (400 mg/kg) anesthesia. The rats were randomly divided into the 4 groups: sham operation (Sham), cerebral ischemia (Isch), combined PreC followed by ischemia (CPreC+Isch) which in its turn were subdivided into: early period of PreC (CPreC + Isch in 1h) and delayed period (CPreC+Isch in 48h). The level of GSK-3 β in brain supernatant was investigated in a day after ischemia by enzyme immunoassay (rat tissue SED317Ra Tests ELISA, Cloud-Clone Corp., USA). Brains were fixed in 10% neutral formalin. Brains' specimens (3,8 \pm 0,2mm posterior to bregma) were prepared with standard protocol of paraffin-embedding, sections 5 μ m in thickness were stained with hematoxylin and eosin and Nissl stain. Normal, reversibly