

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). *Eksperimental'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>

Key words: meniscus, rat, antigen, Soybean agglutinin (SBA).

DOI: 10.29256/v.03.01.2019.escbm41

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

and irreversibly damaged neurons in CA1 and CA3 hippocampus fields were counted at x400 (Carl Zeiss, Germany); necrotic and apoptotic cells were counted separately. The statistical analysis was performed using the software StatPlus 6. For data comparison non-parametric analysis (Kruskal–Wallis (H test) and Mann-Whitney (U test)) were used ($p \leq 0,05$). Data were presented as a median with the 25th and 75th percentiles.

Results. Statistically significant differences in GSK-3 β level were revealed on comparison of the investigated groups ($H=10,26$; $df=3$; $p=0,017$). GSK-3 β level in Shame rat brain was 1,55 (1,50; 1,67) ng/ml. In the Isch group the level of GSK-3 β was reliably higher compared to Shame – 2,0 (1,85; 2,15) ng/ml. The CPreC use before ischemia was associated with decreased GSK-3 β level in brain on the day after the operation. In the group of CPreC + Isch in 1h, GSK-3 β level was 1,16 (0,73; 1,57). In the group of CPreC + Isch in 48h it was 1,25 (1,12; 1,45) ng/ml. In the Isch group the normal pyramidal neurons in hippocampal CA1 and CA3 fields were almost absent with high prevalence of injured neurons ($H=15,41$; $p=0,002$ and $H=21,68$; $p<0,001$ respectively for fields), predominantly necrotic. Besides, amount of apoptotic neurons was significantly higher in both fields compared to Sham group ($U=45,5$; $p=0,042$ for CA1 and $U=6,5$; $p=0,038$ for CA3) and there were less reversibly damaged neurons in CA1 field ($U=46$; $p=0,032$), but their amount didn't differ in CA3 field ($U=32$; $p=0,116$). In groups with the PreC positive morphological changes were detected in comparison with Isch. The number of necrotic neurons decreased in CA3 field in group CPreC + Isch in 1h ($U=2,0$; $p=0,006$) and in group CPreC + Isch in 48h in both fields ($U=44,0$; $p=0,012$ for CA1 and $U=48,5$; $p=0,002$ for CA3). The apoptotic neurons amount decreased significantly as well: in CPreC + Isch in 1h in CA3 field only ($U=0,5$; $p=0,003$); in CPreC + Isch in 48h in both fields ($U=44,0$; $p=0,012$ for CA1 and $U=47,0$; $p=0,004$ for CA3); more protective against neuronal apoptosis was CPreC + Isch in 48h ($U=40,5$; $p=0,005$ for CA1). Also, neurons survived better in PreC groups, especially in CA3 field ($U=41,5$; $p=0,030$ and $U=5,0$; $p=0,012$ for early and delayed PreC) with relatively higher amount of reversibly damaged neurons: in CPreC + Isch in 1h in CA1 ($U=34,5$; $p=0,05$) and CA3 ($U=38,0$; $p=0,015$) fields; in CPreC + Isch in 48h in CA1 ($U=4,0$; $p=0,008$) and CA3 ($U=6,0$; $p=0,018$). Thus, one of possible mechanism of neuroprotection is prevention of opening of nonspecific mitochondria permeability by inhibition of GSK-3 β . This kinase plays the signal role for fulfillment of the neuroprotective effect of CPreC (amtizol+hypobaric hypoxia) confirmed by the morphological examination and may be used as a biomarker of ischemia as well as PreC effectiveness. The use of simpler ELISA GSK-3 β tissue test in comparison with common Western-blot requires further research. Estimation of mechanism of GSK-3 β inhibition in our PreC method, evaluation of phosphorylated GSK-3 β amount, mPTP activity in the brain mitochondrial fraction is planned to be performed.

References:

1. Esposito E., Desai R., Ji X. et al. Pharmacologic pre- and postconditioning for stroke: basic mechanisms and translational opportunity // *Brain Circ.* 2015; 1(1): 104-113. doi: 10.4103/2394-8108.166380
2. Levchenkova O.S. et al. Influence of combined pharmacological and hypoxic preconditioning on animal survival and functional activity of CNS during model cerebral ischemia // *Exp Clin Pharmacol.* 2016; 79(6): 3-8. doi: 10.30906/0869-2092-2016-79-6-3-8.
3. Yang K., Chen Z., Gao J. et al. The key roles of GSK-3 β in regulating mitochondrial activity // *Cell Physiol Biochem.* 2017; 44:1445-1459. doi: 10.1159/000485580
4. Huang S., Wang H., Turlova E. et al. GSK-3 β inhibitor TDZD-8 reduces neonatal hypoxic-ischemic brain injury in mice // *CNS Neurosci Ther.* 2017; 23(5): 405-415. doi: 10.1111/cns.12683.

Key words: glycogen synthase kinase-3 β (GSK-3 β), preconditioning, cerebral ischemia, amtizol.