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Taras Boychuk
Department of Histology, Cytology and Embryology, Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine.

Taras Kmet
Department of Hygiene and Ecology, Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine.

Svitlana Tkachuk
Department of physiology, Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine.

Correspondence
Taras Kmet
Department of Hygiene and Ecology, Higher State Educational Establishment of Ukraine «Bukovinian State Medical University», Chernivtsi, Ukraine.

Reaction of Bcl-2$^+$ cells of the parietal lobe cortex of rats with experimental diabetes mellitus to ischemia-reperfusion

Taras Boychuk, Taras Kmet and Svitlana Tkachuk

Abstract
The peculiarities of anti-apoptotic mechanisms in the nerve and glial cells of the parietal lobe cortex are studied by the density changes of Bcl-2$^+$-cells location and concentration of Bcl-2 protein in them in rats with diabetes mellitus in the dynamics of ischemic-reperfusion cerebral injury.
Bilateral carotid ischemia-reperfusion is found to inhibit anti-apoptotic reaction of nerve cells of the parietal lobe cortex in the early and late post-ischemic periods at the expense of decreased number of Bcl-2$^+$-cells and concentration of Bcl-2 protein in them. In glial cells during the early period of ischemic-reperfusion injury the activity of anti-apoptotic processes intensifies to some extent by means of increased concentration of Bcl-2 protein in them, and on the 12th day the activity of anti-apoptotic processes decrease at the expense of reduced amount of Bcl-2$^+$-cells and the content of Bcl-2 protein in them. Three-month streptozotocin-induced diabetes increases the location density of positive by protein Bcl-2 nerve cells in the parietal lobe cortex without influencing on the location density of Bcl-2$^+$-glial cells and concentration of Bcl-2 protein in them. 20-minute ischemia with one-hour reperfusion in animals with diabetes mellitus considerably enhances anti-apoptotic potential of glial cells in the parietal lobe cerebral cortex at the expense of increased amount of Bcl-2$^+$-cells and the concentration of Bcl-2 protein in them without reliable effect on these indices in nerve cells. On the 12th day of ischemic-reperfusion period in rats with diabetes mellitus, the intensity of anti-apoptotic processes in general decreases both in nerve and glial cells of the parietal lobe cortex.

Keywords: diabetes mellitus, carotid ischemia-reperfusion, parietal lobe cortex, Bcl-2 protein.

1. Introduction
Topicality of the problem of cerebral-vascular pathology is caused by a quick increase of its occurrence and severe consequences [4]. Special attention of scientists is focused on learning ischemic cerebral injuries which constitute over 2/3 of all the cerebral-vascular diseases and are determinative concerning life expectancy and level of health [5]. Diabetes mellitus (DM) occupies an important position among pre-stroke diseases against the ground of which cerebral ischemia develops [6]. DM increases the risk of development of ischemic injuries of neocortex by several times as much [2] and creates a high probability of energy imbalance of the nerve and glial cells [3], eventually resulting in their death.
Nowadays a certain experience of scientific information is accumulated concerning apoptosis of the cerebral cells under conditions of comorbid diabetes and cerebral ischemia-reperfusion [7]. The peculiarities of the reaction of anti-apoptotic Bcl-2 protein in the parietal lobe cortex to a short-term ischemia-reperfusion are studied against the ground of streptozotocin-induced diabetes [1]. Although, literary analysis of this problem did not find any information concerning the condition of anti-apoptotic processes in neocortex of rats with DM complicated by bilateral carotid ischemia-reperfusion in remote post-ischemic period proving the topicality of our study.

1.1 Objective
To study the effect of carotid ischemia with reperfusion of various duration on the reaction of anti-apoptotic Bcl-2$^+$ protein in nerve and glial cells of the parietal lobe cerebral cortex in rats with streptozotocin-induced DM.

2. Materials and methods
In order to design diabetes laboratory 2-month male rats were given single intra-abdominal injection of streptozotocin (Sigma, USA) in the dose of 60 mg/kg of the body weight [8]. Duration of diabetes (since the moment of streptozotocin injection) was three months.

Glycemia level was controlled by means of glucose-oxidase method, and the rats with glucose content more than 10 mmol/L were selected into the experiment. At 5-month age a part of the rats with carbohydrate metabolism disorders as well as control animals of the same age underwent bilateral clamping of the general carotid arteries during 20 minutes under calypsol narcosis (75 mg/kg) [9]. One group of animals was taken out from the experiment in an hour, another group – on the 12th day of post-ischemic period by means of decapitation under narcosis. The brain was removed at cold temperature, the parietal lobe cortex (PLC) was taken by the coordinates of stereotaxic atlas [10] and was placed in Bouin's fluid for 24 hours. After standard histological processing it was filled into paraffin blocks, and histological cuts 5 mcm thick were prepared from them.

The content of Bcl-2 proteion in the nerve and glial cells was detected by means of immunocytofluorescent method. First randomised selected PLC cuts of the cerebral hemispheres were cleaned of paraffin in xylol, then they were rehydrated in descending concentrations of aethanol, and three times for 10 minutes theye were washed in 0,1 M phosphate buffer (pH =7,4). After that rehydrated histological PLC cuts wre placed into the incubator for 18 hours in a moist camera at 4 ºC with primary murine monoclonal antibodies to Bcl-2 of the rat (mouse IgG1 isotype) produced by Sigma Chemical (USA). After washing exessive amount of primary antibodies in 0,1 M phosphate buffer the cuts were placed into the incubator for 60 minutes at 37 ºC with secondary antibodies diluted in the ratio 1:64. Goat antibodies were used as secondary antibodies to murine IgG molecule conjugated with FITC (Sigma Chemical, USA). After incubation the cuts were washed in 0, 1 M phosphate buffer and placed in the mixture of glycerin and phosphate buffer in the ratio 9:1 for further luminescent microscopy. The density of Bcl-2^+-cells location of PLC was studied, the cells were identified by means of fluorescent microscope AXIOSKOP (Zeiss, Germany), as well as general content, concentration of Bcl-2 protein in them, and dispersion of immunoreactive material distribution. The image was filled in the computer system of digital analysis VIDAS-386 (Kontron Elektronik, Germany). Statistical difference value was estimated by t Student criterion for independent sampling. The results are presented as arithmetic mean and standard deviation.

3. Results and Discussion

The results of experimental studies found (Table 1), that after 20-minute ischemia with one-hour reperfusion the total amount of Bcl-2^+-cells in PLC decreased on 21% and location density of Bcl-2^+-nerve cells - on 33%, which is probably indicative of reduced anti-apoptotic potential of nerve cells in PLC; location density of Bcl-2^+-glial cells did not change under this conditions.

On the 12th day of ischemic-reperfusion period a reliable decrease of the total amount of Bcl-2^+-cells and Bcl-2^+-nerve cells was found as compared to the control on 30 and 38% respectively; in addition, reduced density of glial cells on 26% was found in this period as compared to the control. According to our preliminary findings [11] the density of p53$^+$-glial cells in this period increases by 4, 7 times, that in total is the sign of prevailing apoptotic processes in glial cells.

Analysis of percentage ratio of Bcl-2^+-nerve and glial cells demonstrated the absence of reliable changes of this index in the early and late ischemic-reperfusion periods.

Examination of Bcl-2 protein concentration in PLC cells of the cerebral hemispheres in the control animals enabled to detect (Table 2) that 20-minute ischemia with one-hour reperfusion decreased it reliably on 6% in nerve cells and increased on 2% in glial cells. On the 12th day of post-ischemic period the concentration of Bcl-2 protein in nerve cells remained low (on 8%) as compared with that one in the control group of rats. In this term of observation the total content of Bcl-2^+ protein in nerve cells was reliably higher (on 4%) than that of the control, and on 6% - the index of the early period of observation. In spite of the absence of changes of Bcl-2^+ protein concentration in glial cells in this term, its total content decreased on 3% (p<0,05) as compared to the index of animals from the intact group.

In early and late terms of ischemic-reperfusion injury in the examined lobe of the hemispheres dispersion of Bcl-2 protein distribution increased in nerve cells on 18 and 13% respectively, and in glial cells – on 7 and 7% as compared to the control group.

DM modelling caused the increase of the total density of Bcl-2-cells on 27% and Bcl-2^+-nerve cells on 53% as compared to the control group of animals, and it did not effect on the density of Bcl-2^+-glial cells. The changes of percentage ratio of Bcl-2^+-nerve and glial cells in rats with streptozotocin-induced diabetes were in reliable increase of a portion of positive by protein Bcl-2 nerve cells on 23% and decreased portion of Bcl-2^+-glial cells on 17% as compared to the control animals.

Streptozotocin-induced DM resulted in increase of general content of Bcl-2 protein in nerve cells on 3% (p<0, 05) and reduced dispersion of its distribution in glial cells on 6% as compared to the control.

According to literary data the product of bcl-2 gene – Bcl-2 protein is mainly expressed on the mitochondrial membrane, and it is an important repressor of apoptosis as it inactivates pro-apoptotic protein Bax, forms the complex with apoptosis factor Apaf-1 and caspase-9, and blocks further transmission of apoptotic signal [12, 13]. According to our study under conditions of three-month streptozotocin-induced DM in the nerve cells of the examined lobe of neocortex the activation of anti-apoptotic processes occurs.

In animals with DM 20-minute ischemia with one-hour reperfusion increased the total amount of Bcl-2^+-cells and density of Bcl-2^+-glial cells on 30 and 61% respectively as compared to the indices with DM without disorders of cerebral circulation and did not effect on the density of Bcl-2^+-nerve cells. Although, on the 12th day of the post-ischemic period in animals with disorders of carbohydrate metabolism the indices of the total density of Bcl-2^+-cells and density of Bcl-2^+-glial cells returned to the same in animals with DM. Contrary to that, the density of Bcl-2^+-nerve cells decreased on 32 and 26% concerning the indices of DM and early period of the term of observation respectively.

20-minute ischemia/one-hour reperfusion in rats with disorders of carbohydrate metabolism resulted in reduced portion of Bcl-2^+-nerve cells on 29% and increased portion of Bcl-2^+-glial cells on 32%. It is characteristic that to the 12th day of the observation the dynamics of the changes were not found – in this period the portion of nerve cells decreased on 25% and glial cells – on 28% concerning DM.

The concentration of Bcl-2 protein in nerve cells of PLC of animals with DM in the early term of ischemic-reperfusion injury did not change, it increased in the glial cells on 3%; on the 12th day this index decreased on 4% in the nerve cells

concerning DM and on 5% - concerning early term, it remained on the same level in glial cells that corresponds to the early post-ischemic period. The total content of Bcl-2 protein in nerve cells of the examined lobe of the hemisphere of the animals with DM was lower than in animals with DM on 4 and 3 (p<0,05) in the early and late terms respectively.

Therefore, learning the role of anti-apoptotic proteins in the development of neurological diseases against the ground of energy exchange disorders, especially carbohydrate metabolism, can give a valuable information to understand the mechanisms of cerebral neuroplasticity.

Table 1: Density (per 1mm^2) and percentage ratio of Bcl-2$^+$ nerve and glial cells of the parietal lobe cortex of cerebral hemispheres of rats with streptozotocin-induced diabetes mellitus complicated by ischemic-reperfusion cerebral injury (M±m); (n=11)

Group of observation	Total amount Bcl-2$^+$ cells per 1 mm	Density and percentage of Bcl-2$^+$-nerve cells (mm^2)	Density and percentage of Bcl-2$^+$-glial cells (mm^2)
Control	91,12±5,15	34,98±2,73 43,13±3,34	56,14±4,49 56,87±3,34
Ischemia-reperfusion (20 min/1 hour)	71,87±4,92*	23,34±2,18* 39,39±3,84	48,53±4,87 60,61±3,84
Ischemia-reperfusion (12 days)	63,53±5,31*	21,76±2,65* 49,18±6,30	41,77±4,93* 50,81±6,30
Diabetes	116,01±8,12*	53,40±7,66* 52,97±3,06*	62,61±7,72 47,02±3,06*
Diabetes and ischemia-reperfusion (20 min/1 hour)	150,16±12,09#	49,06±5,16 37,50±2,60#	101,10±11,98# 62,50±2,60#
Diabetes and ischemia-reperfusion (12 days)	116,60±8,22&	36,69±3,59#& 39,68±4,06#	76,91±7,65& 60,31±4,06#

Note: *in the numerator of the fraction* – number (density) of appropriate cell classes per 1 mm^2; *in the term of the fraction* – percentage of different classes of cells; difference reliability as compared with the: * - control; ^ - ischemia-reperfusion (20 min / 1 hour) in control animals; # - diabetes; & – ischemia-reperfusion (20 min / 1 hour) in animals with diabetes.

Table 2: Induced diabetes mellitus complicated by ischemic-reperfusion cerebral injury (per 1mm^2) (M±m); (n=11)

Group of observation	Nerve cells			Glial cells		
	Concentration of Bcl-2$^+$protein	Content of Bcl-2$^+$protein	Dispersion of Bcl-2$^+$ protein distribution	Concentration of Bcl-2$^+$protein	Content of Bcl-2$^+$protein	Dispersion of Bcl-2$^+$ protein distribution
Control	0,433±0,005	1,988±0,020	53,28±0,84	0,355±0,002	1,303±0,018	59,58±0,88
Ischemia-reperfusion (20 min/1 hour)	0,406±0,006*	1,950±0,026	62,80±1,47*	0,363±0,003*	1,289±0,019	63,75±0,91*
Ischemia-reperfusion (12 days)	0,397±0,011*	2,075±0,030*^	60,05±1,70*	0,366±0,006	1,259±0,013*	63,53±1,79*
Diabetes	0,440±0,004	2,046±0,017*	51,87±0,79	0,359±0,003	1,281±0,017	55,80±0,95*
Diabetes and ischemia-reperfusion (20 min/1 hour)	0,445±0,005	1,959±0,014#	51,02±0,80	0,368±0,002#	1,285±0,013	54,78±0,58
Diabetes and ischemia-reperfusion (12 days)	0,424±0,007#&	1,997±0,018#	53,35±1,41	0,368±0,003#	1,312±0,029	57,05±1,17

Note: difference probability as compared with: * - control; ^ - ischemia-reperfusion (20 min / 1 hour) in control animals; # - diabetes; & – ischemia-reperfusion (20 min / 1 hour) in animals with diabetes.

4. Conclusions

1. Bilateral carotid ischemia-reperfusion inhibits anti-apoptotic reaction of nerve cells of the parietal lobe cortex in the early and late post-ischemic periods at the expense of decreased number of Bcl-2$^+$-cells and concentration of Bcl-2 protein in them. In glial cells during the early period of ischemic-reperfusion injury the activity of anti-apoptotic processes intensifies to some extent by means of increased concentration of Bcl-2 protein in them, and on the 12th day the activity of anti-apoptotic processes decrease at the expense of reduced amount of Bcl-2$^+$-cells and the content of Bcl-2 protein in them.
2. Three-month streptozotocin-induced diabetes increases the location density of positive by protein Bcl-2 nerve cells in the parietal lobe cortex without influencing on the location density of Bcl-2$^+$-glial cells and concentration of Bcl-2 protein in them.
3. 20-minute ischemia with one-hour reperfusion in animals with diabetes mellitus considerably enhances anti-apoptotic potential of glial cells in the parietal lobe cerebral cortex at the expense of increased amount of Bcl-2$^+$-cells and the concentration of Bcl-2 protein in them without reliable effect on these indices in nerve cells. On the 12th day of ischemic-reperfusion period in rats with diabetes mellitus, the intensity of anti-apoptotic processes in general decreases both in nerve and glial cells of the parietal lobe cortex.

5. References

1. Tkachuk SS, Lenkov AM. Expression of Hif-1α, p53 and Bcl-2 proteins in the brain under the condition of bilateral carotid ischemia-reperfusion in experimental diabetes mellitus in male rats. Clin and experim pathol. 2010; 2(32):111-13.
2. Cheng-guang Song, Xin Yang, Lian-qiu Min, Chang-xi Liu, Chuan-sheng Zhao.The effect of procyanidin on

expression of STAT1 in type 2 diabetes mellitus SD rats with focal cerebral ischemia. Neuroendocrinol Lett. 2014; 35(1):68-72.
3. Li Jing, Li Mai, Jian-Zhong Zhang, Jian-Gang Wang, Yue Chang, Jian-Da Dong *et al.* Diabetes inhibits cerebral ischemia-induced astrocyte activation – an observation in the cingulate cortex. International Journal of Biological Sciences. 2013; 9(9):980-88.
4. Zozulia YP, Mishchenko TS. Problems of vascular-cerebral pathology and the ways of its solution. Journal of the National Academy of Medical Sciences of Ukraine. 2011; 17(1):19-25.
5. Bolacos JP, Moro MA, Lizasoain I, Almeida A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: therapeutic implications. Adv Drug Deliv Rev. 2009; 61:1299-1315.
6. Mendez JD. Trends in advanced glycation end products research in diabetes mellitus and its complications Mol Cell Biochem. 2010; 341(1-2):33-41.
7. Kmet TI. Morphometric characteristics of $p53^{+}$ nerve and glia cells of different cerebral hemisphere lobes in experimental diabetes mellitus complicated by ischemic-reperfusion injuries of the brain. Clin and experim pathol. 2015; 4(54):46-9.
8. Bassirat M. Short- and long-term modulation of microvascular responses in streptozotocin-induced diabetic rats by glycosylated products, J Diabetes Complications. 2008; 22(6):371-6.
9. Skibo GG. The use of various experimental models to study cellular mechanisms of cerebral ischemic lesions Patologija. 2004; 1(1):22-30.
10. Konig JF, Klippel PA. The rat brain. A stereotaxis atlas of forebrain and lower part of the brain stem. Baltimora: The Williams and Wilkins Company, 1963, 162.
11. Kmet TI. Influence of acute circulation disorder in the carotid arteries pool upon $p53^{+}$ protein content in the nervous and glia cells of the cerebral cortex in the parietal lobe of laboratory rats. Vestnik Kaz NMU. 2015; 2:485-7.
12. Hughes P, Bouillet P, Strasser A. Role of Bim and other Bcl-2 family members in autoimmune and degenerative diseases. Curr Dir Autoimmun. 2006; 9:74–94.
13. Brenner C, Cadiou H, Vieira HL, Zamzami N, Marzo I, Xie Z *et al.* Bcl-2 and Bax regulate the channel activity of the mitochondrial adenine nucleotide translocator Oncogene. 2000; 19:329-36.