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DYNAMICS OF CEREBRAL LIPID PEROXIDATION PROCESSES AND ANTIOXIDANT DEFENCE IN RATS WITH STREPTOZOTOCIN-INDUCED DIABETES COMPLICATED BY ISCHEMIC-REPERFUSION LESION OF THE BRAIN

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Abstract

Introduction. The role of oxidative stress in the pathogenesis of ischemic-reperfusion lesions and diabetes mellitus (DM) has been evidenced, although the dynamics of its parameters in case of DM complicated by ischemic-reperfusion lesion of the brain remains uncertain.

The aim of the study. The aim of these studies was to investigate condition of lipid peroxide oxidation and activity of antioxidant enzymes in the neocortex and fields of the hippocampus of male rats with induced diabetes mellitus in the dynamics of incomplete global ischemia-reperfusion of the brain.

Results. In early ischemic-reperfusion period in all the examined structures of the brain without DM the signs of oxidative stress are found which is manifested by accumulation of lipid peroxidation products against the ground of a considerable decrease of superoxide dismutase (SOD) activity. In rats with DM during this period of observation in all the brain structures except CA3 field there are signs of depression of lipid peroxidation processes and activity of antioxidant enzymes.

On the 12^{th} day of ischemic-reperfusion period in rats without DM the content of lipid

peroxidation secondary products in the examined brain structures increases against the ground of reduced activity of antioxidant enzymes which is indicative of increasing signs of oxidative stress in this period. In rats with DM in this term of observation the signs of hyporeactivity of the lipoperoxidation/ antioxidant defence system remain unchanged.

Conclusions. Four-month diabetes mellitus depletes the processes of lipid peroxidation and activity of antioxidant enzymes in the cortex of the frontal and occipital lobes and fields of the hippocampus, and as a result, it inhibits their response to ischemia-reperfusion of the brain both in early and remote post-ischemic periods.

Key words: diabetes mellitus, cerebral ischemia-reperfusion, neocortex, hippocampus, lipid peroxidation, antioxidant enzymes.

According to present views concerning initiation of diabetes mellitus (DM) and its complications oxidative stress possesses a trigger role [1, 2], which is characterized by exhaustion of the cellular system of the antioxidant defence and increased production of free radicals [2, 3]. Meanwhile numerous attempts to apply antioxidant therapy in case of DM still remain less effective (so called “antioxidant paradox”) [4, 5], which is indicative of the necessity to carry out further studies concerning the nature of oxidative stress against this disease with the purpose to improve pathogenetically substantiated means of struggle against formation of complication with underlying diabetes.

Imbalance of pro-oxidative – antioxidant interrelations plays a valuable role in the development of “ischemic” pathobiochemical cascade in case of acute disorders of the cerebral circulation [6, 7]. DM is known to increase the occurrence of ischemic lesions of the brain and aggravate their course [8, 9], although pathogenesis of this combined pathology requires a comprehensive study.

Objective and tasks: to investigate condition of lipid peroxide oxidation and activity of antioxidant enzymes in the neocortex and fields of the hippocampus of male rats with induced diabetes mellitus in the dynamics of incomplete global ischemia-reperfusion of the brain.

Materials and methods of the study. The study was conducted on males of albino nonlinear rats divided into six groups: 1. Control; 2. Rats isolated from the experiment after 20-minute bilateral carotid ischemia with one-hour reperfusion; 3. Rats isolated from the experiment on the 12^{th} day after 20-minute bilateral carotid ischemia; 4. Rats with experimental DM; 5. Rats with DM isolated from the experiment after 20-minute bilateral carotid ischemia with one-hour reperfusion; 6. Rats with DM isolated from the experiment on

the 12th day after 20-minute bilateral carotid ischemia.

DM was simulated by a single intraperitoneal introduction of streptozotocin (Sigma, «Aldrich», 60 mg/kg) to male rats aged 2 months [10]. The period of diabetes with duration of 4 months was considered from the moment of streptozotocin introduction. To imitate incomplete global ischemia-reperfusion of the brain under intraperitoneal narcosis (calypsol, 75 mg/kg) both general carotid arteries were isolated by means of the anterior middle cervical access – they were clipped during 20 minutes and then clips were removed for reperfusion. The animals were taken out from the experiment by means of decapitation under calypsol narcosis. After fixation of the brain in liquid nitrogen using the atlas of stereotactic coordinates [11] the cortex of the frontal and occipital lobes and the hippocampus fields CA1, CA2 and CA3 was taken for the examination. In the homogenates of these structures the content of diene conjugates (DC), Malone dialdehyde (MDA), activity of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPO) was determined [12].

Statistical significance of differences was estimated by Student t-criterion for independent sampling. The findings are presented in the form of arithmetic means and standard deviation.

Results and discussion

Results of the study are presented in Tables 1, 2.

In the cortex of the frontal lobe of animals without DM 20-minute ischemia with one-hour reperfusion resulted in 10, 37, 63 and 99% increased contents of DC, MDA, activity of catalase and GPO respectively against the ground of 32% reduced activity of SOD. On the 12th day of post-ischemic period 28 and 51% reduction of DC and SOD was registered, and 35 and 27% decrease – concerning early ischemic-reperfusion period. At the same time activity of GPO in this term increased as compared to the parameters of the control animals, and concerning the early period – activity of GPO became 58% as much and activity of catalase became 20% less.

In the cortex of the occipital lobe of animals of this experimental group in early post-ischemic period there were changes of the following character: increased content of MDA (24 %), activity of catalase (43%) and 2,4 times GPO, and 34% decrease of SOD activity. In late ischemic-reperfusion period the content of MDA increased (37%) concerning the control group and SOD activity decreased in 2,2 times. The dynamics was connected with 10% increase of MDA content as compared to the previous term and decreased activity of SOD, catalase and GPO 50 and 22% as less and in 3,7 times respectively. Therefore, the response of the examined lobes of the cortex by the investigated parameters is more unique in early post-

ischemic period and differs considerably – in the late one.

Table 1

Parameters of intensity of lipid peroxidation and activity of enzymes of antioxidant defence in the neocortex of male rats with experimental diabetes mellitus in the dynamics of incomplete global ischemia-reperfusion of the brain (M±m, n=11)

Experimental group	DC (nmol/mg of protein)	MDA (nmol/mg of protein)	SOD (units/min of mg of protein)	Catalase (mcmol/mof protein)	GPO (nmolG-SH min mg of protein)
Cortex of the frontal lobe					
Control	2,75±0,09	0,519±0,015	38,64±0,92	2,49±0,42	0,520±0,101
Ischemia-reperfusion 20 min / 1 hour	3,04±0,08 $p_1<0,05$	0,712±0,031 $p_1<0,001$	26,22±0,69 $p_1<0,001$	4,06±0,15 $p_1<0,005$	1,035±0,097 $p_1<0,005$
Ischemia-reperfusion 12 days	1,98±0,07 $p_1<0,001$ $p_2<0,001$	0,769±0,020 $p_1<0,001$ $p_2<0,05$	19,06±0,52 $p_1<0,001$ $p_2<0,001$	3,27±0,22 $p_2<0,01$	1,639±0,101 $p_1<0,001$ $p_2<0,005$
Diabetes	1,60±0,10 $p_1<0,001$	0,509±0,031	7,28±0,61 $p_1<0,001$	4,27±0,33 $p_1<0,005$	0,512±0,010
Diabetes and ischemia-reperfusion 20 min / 1 hour	1,77±0,13	0,414±0,034 $p_3<0,05$	6,16±0,28	1,74±0,15 $p_3<0,001$	0,335±0,021 $p_3<0,001$
Diabetes and ischemia-reperfusion 12 days	1,68±0,07	0,448±0,029	4,02±0,38 $p_3<0,005$ $p_4<0,005$	2,15±0,11 $p_3<0,001$ $p_4<0,05$	0,356±0,027 $p_3<0,005$
Cortex of the occipital lobe					
Control	2,88±0,06	0,592±0,017	36,34±1,03	2,58±0,33	0,498±0,101
Ischemia-reperfusion 20 min / 1 hour	3,15±0,12	0,738±0,021 $p_1<0,001$	23,80±0,62 $p_1<0,001$	3,70±0,16 $p_1<0,01$	1,210±0,111 $p_1<0,001$
Ischemia-reperfusion 12 days	2,75±0,11 $p_2<0,05$	0,812±0,019 $p_1<0,001$ $p_2<0,05$	16,23±0,42 $p_1<0,001$ $p_2<0,001$	2,87±0,12 $p_2<0,005$	0,326±0,091 $p_2<0,001$
Diabetes	1,48±0,123 $p_1<0,0005$	0,443±0,019 $p_1<0,0005$	6,54±0,35 $p_1<0,0005$	3,32±0,28	0,464±0,017
Diabetes and ischemia-reperfusion 20 min / 1 hour	1,40±0,106	0,308±0,041 $p_3<0,01$	3,91±0,37 $p_3<0,001$	1,32±0,12 $p_3<0,001$	0,320±0,041 $p_3<0,005$
Diabetes and ischemia-reperfusion 12 days	1,29±0,113	0,521±0,048 $p_4<0,005$	3,29±0,21 $p_3<0,001$	1,86±0,10 $p_3<0,001$ $p_4<0,005$	0,392±0,039

Notes: this Table and the following Table contains: probability of differences as compared to: p – control; p_1 – ischemia-reperfusion (20 min / 1 hour) in the control animals; p_3 – diabetes; p_4 – ischemia-reperfusion (20 min / 1 hour) in animals with diabetes.

Table 2

Intensity of lipid peroxidation and activity of the antioxidant defence activityin the hippocampus of male rats with experimental diabetes mellitus in the dynamics of incomplete global ischemia-reperfusion of the brain (M±m, n=11)

Group of observation	DC (nmol/mg of protein)	MDA (nmol/mg of protein)	SOD (units/min mg of protein)	Catalase (mcmol/ mg of protein)	GPO (nmolG-SH min mg of protein)
Hippocampus field CA1					
Control	3,21±0,12	0,701±0,010	47,38±1,38	3,09±0,26	0,758±0,108
Ischemia-reperfusion 20 min / 1 hour	2,75±0,12	0,765±0,017 $p_1<0,05$	20,58±0,52 $p_1<0,001$	2,96±0,15	0,816±0,111
Ischemia-reperfusion 12 days	1,89±0,16 $p_1<0,001$ $p_2<0,005$	0,789±0,023 $p_1<0,005$	16,52±0,48 $p_1<0,001$ $p_2<0,001$	2,28±0,11$p_1<0,01$ $p_2<0,005$	0,798±0,093
Diabetes	1,61±0,13 $p_1<0,001$	0,436±0,027 $p_1<0,001$	6,26±0,29 $p_1<0,001$	4,01±0,29	0,490±0,046 $p_1<0,05$
Diabetes and ischemia-reperfusion 20 min / 1 hour	2,12±0,26	0,447±0,043	8,65±1,31	2,02±0,16 $p_3<0,001$	0,454±0,049
Diabetes and ischemia-reperfusion 12 days	2,01±0,19	0,396±0,033	7,79±0,84	2,48±0,26 $p_3<0,005$	0,389±0,031
Hippocampus field CA2					
Control	2,93±0,11	0,668±0,011	43,26±1,27	2,67±0,06	0,734±0,084
Ischemia-reperfusion 20 min / 1 hour	2,26±0,09	0,769±0,019 $p_1<0,01$	20,31±0,28 $p_1<0,001$	3,96±0,17 $p_1<0,001$	0,844±0,088
Ischemia-reperfusion 12 days	3,49±0,12 $p_1<0,005$ $p_2<0,001$	0,797±0,013 $p_1<0,001$	19,16±0,20 $p_1<0,001$ $p_2<0,01$	2,78±0,13 $p_2<0,005$	0,692±0,053
Diabetes	1,57±0,12 $p_1<0,001$	0,476±0,032 $p_1<0,001$	7,45±0,41 $p_1<0,001$	3,52±0,31 $p_1<0,025$	0,468±0,042
Diabetes and ischemia-reperfusion 20 min / 1 hour	2,14±0,31	0,485±0,071	6,77±0,94	1,45±0,16 $p_3<0,001$	0,424±0,031
Diabetes and ischemia-reperfusion 12 days	1,69±0,11	0,398±0,065	5,68±0,56	2,29±0,20 $p_3<0,005$ $p_4<0,01$	0,379±0,033
Hippocampus field CA3					
Control	3,14±0,09	0,701±0,017	43,77±0,68	3,25±0,09	0,738±0,093
Ischemia-reperfusion 20 min / 1 hour	3,35±0,09	0,790±0,015 $p_1<0,005$	20,40±0,79 $p_1<0,001$	4,10±0,10 $p_1<0,001$	0,908±0,083
Ischemia-reperfusion 12 days	2,96±0,09 $p_2<0,01$	0,822±0,018 $p_1<0,005$	22,16±0,53 $p_1<0,001$	4,76±0,12 $p_1<0,001$ $p_1<0,005$	0,979±0,083
Diabetes	1,41±0,08 $p_1<0,001$	0,459±0,011 $p_1<0,001$	7,56±0,61 $p_1<0,001$	3,56±0,35	0,439±0,018
Diabetes and ischemia-reperfusion 20 min / 1 hour	2,16±0,11 $p_3<0,001$	0,523±0,029	6,32±0,50	2,28±0,28 $p_3<0,05$	0,361±0,026 $p_3<0,05$
Diabetes and ischemia-reperfusion 12 days	1,59±0,09 $p_4<0,001$	0,508±0,019	7,06±0,50	2,67±0,22 $p_3<0,05$	0,328±0,036 $p_3<0,001$

As to the hippocampus fields higher content of MDA (9, 15, 13%) and lower activity of SOD (in 2,3; 2,1 and 2,1 times) were found in early period of the study in rats without DM in the fields CA1, CA2, CA3 respectively. Higher activity of catalase was found in the fields CA2 and CA3 – 50 and 25% respectively.

On the 12th day of the study the content of MDA concerning the control in the fields of CA1, CA2 and CA3 remained 13, 19 and 17% higher respectively; SOD activity was lower (in 2,9, 2,3, 1,8 times). In addition, in this period of time a lower content of DC and catalase was found concerning the control and early period (70 and 31%; 26 and 23% respectively) in the field CA1; in the field CA2 – higher content of DC (19 % concerning the control and 54 % – concerning the previous term), lower catalase concerning the index of the early period (30%); in the field CA3– higher catalase content (50 % concerning the control and 16 % – concerning the previous term) and lower – DC concerning the early period (22 %).

The findings presented are indicative of oxidative stress occurrence in all the examined lobes of the brain after 20-minute ischemia-one hour reperfusion. At this period in the neocortex and hippocampus fields CA2 and CA3 LPO intensity and activity of antioxidant defence enzymes increase parallel (except SOD activity), in the field CA1 MDA content increased against the ground of reduced activity of catalase and GPO. A stable, more intensive with time decrease of SOD activity is found in animals of all the experimental groups.

The parameters found do not change in none of the structures examined till the 12th day of ischemic-reperfusion period which is indicative of extension of pathological changes initiated by ischemia-reperfusion.

Peculiarity of the pro-oxidant-antioxidant interrelations at this time is the fact that LPO occurs against the ground of depression of antioxidant enzymatic defence in all the structures except the cortex of the frontal lobe and CA3 field of the hippocampus where the signs of restoration (stabilization) of the balance in the lipid peroxidation/antioxidant defence system are found.

Generally accepted point of view is availability of oxidative stress under conditions of diabetes mellitus initiating key ways of complications of diabetes mellitus [1, 2]. Although the results obtained in our studies, demonstrated a decreased content of two or one LPO products in all the structures against the ground of a reduced activity of the antioxidant enzymes. Thus, in the cortex of the frontal lobe we have found lower content of DC (42%) and SOD activity (in 5,3 times) as compared to the parameters in animals of the control groups. At the same time catalase activity was in 1,7 times higher.

In the cortex of the occipital lobe, fields of the hippocampus CA1, CA2, CA3 a lower content of DC and MDA was found 49 and 25% as much, in 2,0 and 1,6 times; 90 and 29%, in 2,2 and 1,5 times respectively; lower activity of SOD in 5,5, 7,6, 5,8 and 5,8 times respectively. In addition, in the fields CA1 and CA2 GPO activity was in 1,5 and 1,6 times lower. Thus, the ratio of reduced activity of antioxidant enzymes, and SOD in particular, was considerably higher than the ratio of a reduced content of lipid peroxidation products, which is indicative of exhaustion of the antioxidant potential.

Analysis of the character of response of the indicated parameters on ischemia-reperfusion in animals with DM showed considerable differences from that one in animals without diabetes. Thus, after 20-minute ischemia and one hour reperfusion in the cortex of the frontal and occipital lobes 19 and 30% reduced content of MDA was found as compared to the parameters in animals with DM without disorders of the cerebral circulation. At the same time in the cortex of the frontal lobe the activity of catalase and GPO reduced in 2,5 and 1,5 times, and in the cortex of the occipital lobe – activity of SOD, catalase and GPO in 1,7, 2,2 and 1,5 times respectively. In the hippocampus fields CA1 and CA2 of animals of this experimental group only reduced activity of catalase in 2,0 and 2,4 times was found, and in CA3 field – increased content of DC in 1,5 times with parallel decrease of the activity of catalase and GPO 36 and 18% as much.

On the 12^{th} day of ischemic-reperfusion period in the cortex of the frontal lobe of animals with DM as compared to the parameters under conditions of DM without cerebral disorders activity of SOD, catalase and GPO decreased in 1,8, 2,0 and 1,4 times against the ground of unchanged content of LPO products. In comparison with the previous term catalase activity was 23% higher, and SOD activity – in 1,5 times lower.

In the cortex of the occipital lobe of rats of this experimental group as compared to the parameters in animals with DM without disorders of the cerebral circulation SOD activity was twice as less and catalase activity – in 1,8 times less, and in comparison with early post-ischemic period MDA content was 69% higher, that practically was equal to that one in case of diabetes, and catalase activity increased in 1,4 times.

In the hippocampus field CA1 of animals of the indicated experimental group in comparison with the parameters of diabetes without simulating ischemia-reperfusion catalase activity decreased in 1,6 times; in the field CA2 catalase activity decreased in 1,5 times. Although it should be noted that concerning the previous period this parameter increased in 1,6 times; in the field CA3 – the content of DC, activity of catalase and GPO decreased 26, 25, 25 % as much.

Conclusions. 1. In early ischemic-reperfusion period in all the examined structures of the brain in rats without DM there are signs of oxidative stress which is manifested by accumulation of lipid peroxidation products against the ground of a considerable decrease of SOD activity. In rats with DM in this term of observation in all the structures of the brain except CA3 field there are signs of depression of lipid peroxidation processes and activity of antioxidant enzymes.

2. On the 12^{th} day of ischemic-reperfusion period in rats without DM decrease of MDA content in the examined structures of the brain occurs against the ground of reduced activity of antioxidant enzymes which is indicative of intensification of oxidative stress in this period. In rats with DM at this term of observation the signs of hyporeactivity of the lipid peroxidation/antioxidant defence system remain.

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