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MODIFYING EFFECT OF DIABETES MELLITUS ON THE DYNAMICS OF PARAMETERS OF FREE RADICAL PROCESS INTENSITY AND ANTIOXIDANT PROTECTION ACTIVITY IN THE LIVER OF RATS WITH INCOMPLETE GLOBAL CEREBRAL ISCHEMIA-REPERFUSION

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Abstract

According to the WHO expert assessment diabetes mellitus (DM) will take the seventh position among leading causes of death in 2030 [1]. The most dangerous complications of DM becoming a cause of severe disability or mortality are acute cerebral disorders of ischemic genesis [2, 3], which in case of this pathology occur several times more frequently than in case of its absence [4, 5]. The causes of complicated development of cerebral ischemia-reperfusion against the ground of DM are being investigated actively, and pathogenesis of brain damage in case of this comorbid pathology appears to be the most studied, although the mechanisms of morphofunctional disorders of the parenchymal organs remain without attention of researchers, since they are touched upon only in several works [6, 7]. Meanwhile, even short hypoxic conditions of the brain are known to result in considerable pathohistological changes in the internal organs becoming a cause of disorders in their functional state and require correction [7, 8]. The most permanent and long changes occur in the liver even to the destruction of separate cells or their groups [9-11]. These changes in case

of cerebral ischemia are initiated due to redistribution of blood flow and oxygen supply in favour of the brain, and therefore partial oxygen pressure in the abdominal organs decreases, and in the liver in particular [10].

From the other hand, metabolic disorders associated with diabetes mellitus are generally known to cause liver damage [12-14], therefore diabetic complications caused by cerebral ischemia-reperfusion should be logically expected to intensify morphofuncitonal changes in the organ. Meanwhile, we have not found investigations of the kind described in the scientific literature yet.

Objective and tasks: to examine the signs of oxidative and nitrosative stress in the liver of rats with diabetes mellitus complicated by cerebral ischemia-reperfusion.

In the liver of rats without DM 20 minute carotid ischemia with 1 hour reperfusion by the changes of the content of lipid peroxidation products and activity of antioxidant enzymes results in depression of the lipid peroxidation-antioxidant protection system. On the 12^{th} day of observation diene conjugated content increases in the organ against the ground of increased activity of superoxide dismutase and glutathione peroxidase, which in general is indicative of a compensatory reaction character of the system. The indices of protein oxidative modification and nitrogen oxide metabolism in the liver of animals without diabetes at the early post-ischemic period remain unchanged, and on the 12^{th} day of the experiment they increase, which confirms availability of oxidative and nitrosative stress at this period. With DM available reaction of all the above indices in the liver is absent both at the early and late post-ischemic periods, which characterizes a-reactivity of these biochemical parameters concerning cerebral ischemia-reperfusion.

Key words: diabetes mellitus, antioxidant protection, global cerebral ischemia-reperfusion

According to the WHO expert assessment diabetes mellitus (DM) will take the seventh position among leading causes of death in 2030 [1]. The most dangerous complications of DM becoming a cause of severe disability or mortality are acute cerebral disorders of ischemic genesis [2, 3], which in case of this pathology occur several times more frequently than in case of its absence [4, 5]. The causes of complicated development of cerebral ischemia-reperfusion against the ground of DM are being investigated actively, and pathogenesis of brain damage in case of this comorbid pathology appears to be the most studied, although the mechanisms of morphofunctional disorders of the parenchymal organs

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Materials and methods of the study. Non-linear albino laboratory male rats were used in the experiment. Diabetes mellitus was simulated by a single intraperitoneal administration of streptozotocin (Sigma, USA, 60 mg per 1 kg of the body weight) [6]. Glycemia level was determined by means of glucose oxidase method. Duration of diabetes was four months. Incomplete global cerebral ischemia was modeled in apart of control rats by means of bilateral clipping of the common carotid arteries during 20 minutes. The results were assessed after 20-minute carotid ischemia with one-hour reperfusion and on the 12^{th} day of the post-ischemic period.

The control group included false operated rats without and with diabetes mellitus experiencing all the manipulations till the stage of blood flow arrest along the carotid arteries. Euthanasia of animals was made by means of decapitation under narcosis.

In the liver homogenates the following was determined: the content of dienes conjugated (DC), Malone aldehyde (MA), products of protein oxidative modification (POM) of a neutral and basic character, nitrogen oxide metabolites [7]. Antioxidant protection state was assessed by the activity of superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPO) [15].

The results of the study are processed by means of the packet of the applied programs “Statistica (“Statsoft”, USA). The groups of comparison were distributed adequately by Shapiro-Wilk test. Statistical value of differences was evaluated by Student t-criterion for

independent sampling. Differences were considered reliable with probability of null hypothesis less than 5% ($p<0,05$).

Results and discussion. In the liver of animals without diabetes mellitus 20-minute ischemia with one-hour reperfusion caused reliable decrease of DC, MA and catalase content compared with the same indices in rats from the control group (Table 1). On the 12^{th} day of ischemic-reperfusion period DC content increased in the organ without diabetes mellitus both in the animals from the control group and those after early post-ischemic period. Moreover, at this period the activity of SOD and GPO increased reliably and catalase activity decreased in comparison with the parameters of the control group; activity of the first two antioxidant enzymes increased as well concerning early post-ischemic period.

Table 1 – Indices of lipid peroxidation intensity and antioxidant protection enzymatic activity in the liver of rats with experimental diabetes mellitus in the dynamics of incomplete global cerebral ischemia-reperfusion (M±m, n=11)

Group of observation	DC (nmol/mg of protein)	MA (nmol/mg of protein)	СОД (units/min. mg of protein)	Catalase (mcmol/mg of protein)	GPO (nmolG-SH min. mg of protein)
Control	2,14±0,13	0,810±0,039	13,18±0,85	46,81±2,41	0,571±0,027
20 minute ischemia 1 hour reperfusion	1,69±0,16 p<0,05	0,674±0,043 p<0,05	12,55±1,26	38,49±2,38 p<0,05	0,511±0,040
12^{th} day of post-ischemic period	2,98±0,21 p<0,01 p_1<0,005	0,759±0,037	15,98±0,86 p<0,01 p_1<0,01	40,47±1,38 p<0,05	0,762±0,037 p<0,001 p_1<0,001
Diabetes	1,58±0,15 p<0,05	0,561±0,051 p<0,005	9,57±0,75 p<0,01	32,36±2,18 p<0,001	0,416±0,040 p<0,01
Diabetes and 20 minute ischemia and 1 hour reperfusion	1,78±0,29	0,644±0,046	10,67±1,25	36,51±3,52	0,448±0,039
Diabetes and ischemia-reperfusion 12 days	1,86±0,16	0,598±0,036	8,75±1,55	29,98±1,38	0,337±0,045

Notes. Here and in the following Table: reliability of difference compared with: p – control; p_1 – ischemia-reperfusion (20 min / 1 hour) in control animals.

Thus, it can be suggested that even after 20 minute cerebral ischemia with 1 hour reperfusion the reaction occurs in the system of lipid peroxidation-antioxidant liver protection with the signs of reduced level of its functional activity, which on the 12^{th} day of the study changes into the activation of this system components. Parallel growth of both DC content and activity of antioxidant enzymes is indicative of a compensatory character of this reaction.

In the liver of rats with DM reliably lower levels of DC, MA, activity of SOD, catalase and GPO are determined compared with the animals from the control group. These changes can be indicative of exhausted prooxidant-antioxidant reserves of the liver at this term of diabetes. This opinion is confirmed while modeling cerebral ischemia-reperfusion in rats with DM – both at the early and late ischemic-reperfusion periods reliable changes of the examined parameters of lipid peroxidation and antioxidant protection are not found concerning similar ones in animals with diabetes non-complicated by cerebral ischemia-reperfusion.

The results of examination of POM products and nitrogen oxide metabolites in the liver of animals from different experimental groups are presented in Table 2.

Table 2 – Content of protein oxidative modification in the liver of rats with diabetes mellitus complicated by incomplete global cerebral ischemia-reperfusion (M±m, n=11)

Group of observation	Content of NO metabolites (NOx, mcmol/L)	Content of aldehyde and ketone derivatives of	
		neutral character (o.d.u./g of protein, 370 nm)	basic character (o.d.u./g of protein, 420 nm)
Control	81,88±3,78	35,39±1,51	17,83±0,54
Cerebral ischemia-reperfusion (20 min/ 1 hour)	80,18±4,23	35,09±2,83	16,95±0,82
Cerebral ischemia-reperfusion (12 days)	92,12±2,16 $p<0,05$ $p_1<0,01$	39,52±0,98 $p<0,05$	19,81±0,31 $p<0,01$ $p_1<0,01$
Diabetes	93,89±2,29 $p<0,01$	29,92±2,31	15,47±0,81 $p<0,05$
Diabetes and cerebral ischemia-reperfusion (20 min/1 hour)	93,27±6,89	32,57±6,23	17,47±2,78
Diabetes and cerebral ischemia-reperfusion (12 days)	89,65±1,87	28,45±0,71	16,44±1,48

Notes. o.d.u. – optic density units.

In the liver of animals without DM after 20 minute ischemia and 1 hour reperfusion reliable changes of the above parameters are not found. On the 12th day of ischemic-reperfusion period concerning the indices in the control group of animals the products of POM of a neutral and basic character, the content of nitrogen monoxide metabolites increased reliably, which is indicative of oxidative and nitrosative stress. The dynamics of the content of POM products of a neutral character was not found, but the content of POM products of a basic character and nitrogen oxide metabolites appeared to be higher than that of the early term of observation.

In the liver of rats with diabetes lower content of POM products of a neutral and basic character, and higher content of nitrogen oxide metabolites were determined in comparison with animals from the control group.

A reliable reaction of the above indices in rats with diabetes under conditions of early and late ischemic-reperfusion periods was not determined.

On the whole these results are indicative of the fact that DM eliminates reaction of all the examined liver parameters on cerebral ischemia-reperfusion. The causes of such "biochemical a-reactivity" require investigation, although it can be suggested that it is stipulated by exhaustion of metabolic reserves by the underlying disease.

Conclusions:

1. In the liver of rats without DM 20 minute carotid ischemia with 1 hour reperfusion by the changes of the content of lipid peroxidation products and activity of antioxidant enzymes results in depression of the lipid peroxidation-antioxidant protection system. On the 12th day of observation diene conjugated content increases in the organ against the ground of increased activity of superoxide dismutase and glutathione peroxidase, which in general is indicative of a compensatory reaction character of the system.

2. The indices of protein oxidative modification and nitrogen oxide metabolism in the liver of animals without diabetes at the early post-ischemic period remain unchanged, and on the 12th day of the experiment they increase, which confirms availability of oxidative and nitrosative stress at this period.

3. With DM available reaction of all the above indices in the liver is absent both at the early and late post-ischemic periods, which characterizes a-reactivity of these biochemical parameters concerning cerebral ischemia-reperfusion.

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