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Experimental evaluation of Enalapril on the antioxidant protection and nitrogen oxide system of the brain in rats with type 2 diabetes mellitus

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ABSTRACT

Enalapril effect produced on the antioxidant protection and nitrogen oxide system in the brain of rats with experimental type 2 diabetes mellitus is examined. The experiments were conducted on nonlinear laboratory albino male rats with 0.18-0.20 kg of their body weight and type 2 diabetes mellitus simulated by streptozotocin and high-fat diet. Intensity of lipid peroxide oxidation was evaluated by the content of products reacting with 2-thiobarbituric acid. The state of the antioxidant protection system was evaluated by the activity of superoxide dismutase and catalase. To evaluate the state of NO-system in the cerebral cortex and hippocampus, the content of stable nitrogen monoxide metabolites was determined: nitrite-ions as well as activity of NO-synthase. Under conditions of damaged nervous system induced by type 2 diabetes mellitus the content of products reacting with 2thiobarbituric acid in the cerebral cortex and hippocampus is found to increase and the activity of catalase and superoxide dismutase is found to decrease; the content of nitrite-ions and NO-synthase activity increases which is indicative of intensification of lipid peroxide oxidation processes and inhibition of the antioxidant protection and nitrogen oxide systems. Under effect of enalapril (14 days) rats with type 2 diabetes mellitus demonstrate the following in both examined structures of the brain: the content of products reacting with 2-thiobarbituric acid decreases, activity of catalase and superoxide dismutase increases in the cerebral cortex, the content of nitrite-ions in both examined structures of brain decreases, and activity of NO-synthase decreases in the hippocampus only. The obtained results are indicative of a correcting effect of enalapril on the prooxidant-antioxidant balance, and moreover, on the indices of NO system in the cerebral cortex and hippocampus of rats with nervous system damage, which evidences its available neuroprotective properties with central genesis complications due to type 2 diabetes mellitus.

Keywords: type 2 diabetes mellitus, enalapril, nitrogen oxide systems, superoxide dismutase, catalase.

1. INTRODUCTION

Diabetes mellitus (DM) is the most spread endocrine pathology among able to work population. Due to improvement of tendencies of therapy, methods of control and correction of hyperglycemia, the life expectancy of such patients increased. Unfortunately, amount of DM complications, including degenerative changes in the central nervous system (CNS), increases. Irrespective of causes promoting development of neurodegeneration, a pathogenic component of death of the brain cells is oxidative stress, an increased level of endogenous factors of inflammation etc. It should be noted that in pathogenesis of type 2 DM insulin resistance is of a special value. Its targets are adipose, hepatic, muscular, and endothelial tissues. Their condition is associated with glucose metabolism. Blood supply of the brain is known to depend on a functional state of the vascular endothelium – a regulating organ of a proper homeostatic balance supply. In patients with DM vasoconstriction prevails over vasodilation. It is caused by an excessive activation of the sympathoadrenal and renin-angiotensin systems (RAS) (Rajendran et al., 2013).

Moreover, free radicals are known to play a leading role in pathogenesis of DM and its complications (Ullah et al., 2016). Thus, glucose oxidation results in the formation of oxygen active forms inducing the processes of oxidation and destruction of biological molecules, namely, the neurons of the brain and hippocampus. In case of pronounced and long activation of lipid peroxide oxidation (LPO) processes endogenous antioxidants become exhausted. Their compensatory supply slows down due to physical properties of the cellular membranes (Michael & Brent, 2017). Under conditions of hyperproduction of free radicals and reduced antioxidant protection nitrogen oxide (NO) synthesis increases. It promotes formation of peroxinitrite, a powerful vasoconstrictor. Undoubtedly, a high frequency of metabolic DM complications is caused by rebuilding of the macro- and microcirculatory stream condition from compensatory-adjacent to pathological character. Post-ischemic damage of the cerebral tissue occurs against the ground of typical vascular disorders.

The systemic, tissue and cerebral RAS proper are known to be activated with DM and involved to pathogenic mechanisms of the structural-functional changes from the side of the CNS. Therefore, pharmacological correction of RAS state is a target therapy of brain damage with DM. Moreover, RAS effector – angiotensin II (AII) – is a regulator of insulin secretion by the pancreatic β -cells and tissue sensitivity to insulin. Angiotensin converting enzyme inhibitors (ACEI) are known to reduce the production of NADPH-oxidase complex – an important intracellular source of oxygen active forms (Abadi et al., 2018). Inhibition of AII formation with enalapril under condition of diabetic lesion of the CNS might be reflected by biochemical changes in the cerebral cortex and hippocampus. Objective of the work is to study enalapril effect on the antioxidant protection state and the indices of nitrogen oxide system in the cerebral cortex and hippocampus under conditions of experimental lesion of the CNS due to type 2 DM.

2. MATERIAL AND METHODS

The experiments were conducted on male rats with the body weight of 0.18-0.20 kg, kept under conditions of natural changes of day and night. All the experimental procedures were made keeping to the requirements of the Council of Europe Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (18.03.1986); Council Directive 86/609/EEC of 24 November 1986 on the approximation of laws, regulations and administrative provisions of the Member States regarding the protection of animals used for experimental and other scientific purposes; the Order of the Ministry of Health of Ukraine Nº 690 of 23.09.2009. In addition, they were confirmed by the Board on Biomedical Ethics Issues at the Higher State Educational Establishment of Ukraine «Bukovinian State Medical University».

Type 2 DM was modeled by means of intraperitoneal injection of streptozotocin ((Stz); Sigma, USA) in a single dose of 30 mg/kg given to rats preliminary kept during 30 days on high-fat diet with a free access to fructose solution (200 g/L) (Jurgoński et al., 2014; Damasceno et al., 2014; Kmet et al., 2019). The control group of animals with a standard diet and free access to water was injected with the solvent only – citrate buffer (pH=4.5). On the 7th day after Stz injection type 2 DM was evidenced by the detection of glucose concentration in the blood plasma on an empty stomach. Rats with hyperglycemia lower than 10 mmol/L were excluded from the experiment. On 11th week after Stz injection rats with type 2 DM were randomized into two groups: 1 – with saline injection; 2 – with intraperitoneal enalapril injection in the dose of 1 mg/kg (14 days); the control group received saline.

Euthanasia of rats was performed under light ether narcosis. The brain was removed cold, carefully washed with cooled 0.9 % NaCl solution, and the cerebral cortex and hippocampus were isolated by the stereotaxic atlas (Paxinos & Watson, 2013). Cytoplasmic fraction was isolated by means of the method of differential centrifugation of homogenate of the examined structures on the refrigerator centrifuge with 1000 g per 10 min., followed by 1400 g per 10 min. at the temperature of 4°C. LPO intensity was evaluated by the content of products reacting with 2-thiobarbituric acid (TBA) (Kushnir et al., 2018). The amount of TBA was calculated in micromole per 1 gram of tissue. The state of the antioxidant protection system was evaluated by the activity of superoxide dismutase (SOD) [EC 1.15.1.1] (Dubinina et al., 1983) and catalase [EC 1.11.1.6] (Korolyuk et al., 1988). To evaluate the state of NO system in the cerebral cortex and hippocampus the content of stable nitrogen monoxide was determined – nitrite-anions (NO₂) – by means of Griess method, and NO-synthase (NOS) activity [EC 1.14.13.39] by means of the spectrophotometric methods (Chekman et al., 2016). The amount of protein in the sample was determined by means of Lowry method (Ceban et al., 2016).

The results of the study were statistically processed by means of Student t-criterion. Distribution of values in samples was preliminary checked in order to prove an adequate method of statistical assessment of a mean difference between the groups of the study. According to Shapiro-Wilk criterion the data concerning distribution deviation in samples from that of the norm were not obtained (p>0.05). Taking into account the above mentioned application of Student t-criterion was considered to be sufficient to obtain valid conclusions. At the same time, to prove reliability of conclusions Mann-Whitney non-parametric comparison criterion was applied, which showed similar results of calculations by means of Student t-criterion concerning p value. Therefore, $p \le 0.05$ was considered to be a sufficient level of discrepancy probability.

3. RESULTS

Glucose metabolism disorder is one of the major causes of damage of the CNS in DM. Glucose in the blood is a source of energy, and mitochondria are the most important place for glucose aerobic oxidation in the brain. Thus, on the 10th week after Stz injection glucose level in rats was 11.99±1.562 mmol/L, against 4.87±0.713 mmol/L in the control. Attention is paid to the fact that in comparison with the group without correction, on the 14th day of enalapril administration reliable changes of the parameter were not found. Thus, glucose concentration in this rats was 10.92±0.976 mmol/L (Fig. 1). Thereafter, a chronic stable increase of glucose level in our study promotes increase of the amount of oxygen active forms and activates oxidative stress which produces a negative effect on the brain function. We have determined (Table 1) that oxidative stress occurs in the CNS of rats with type 2 DM manifested by an increased amount of TBA in the cerebral cortex and hippocampus. Thus, in comparison with the control group TBA content increased as much as twice in the examined structures. Moreover, in rats with DM activity of the antioxidant protection enzymes decreased – SOD and catalase: 1.9 and 1.5 times – in the cerebral cortex, and 1.4 and 1.6 times – in the hippocampus respectively.

It should be added that chronic increase of free radicals level results in the induction of a cascade of so-called stress-sensitive NO signal way, which according to the latest data is involved to pathogenesis of type 2 DM and its complications (Xiaoxue et al., 2019). In rats with type 2 DM an increased NO₂ content was found: in the cerebral cortex -2.5 times, in the hippocampus -2.8 times (Fig. 2). At the same time, NOS activity in both examined structures 1.8 times increased on an average (Fig. 3).



Figure 1 Glucose concentration in the blood of rats ($M \pm M$, n=7) Notes: * – reliability of differences in comparison with the control group of animals



Figure 2 Effect of enalapril on the content of nitrite-anions in the cytosolic fraction of the brain and hippocampus of rats with type 2 diabetes mellitus ($M \pm m$, n=7)

- Notes: * reliability of differences in comparison with the control group of animals,
 - ** reliability of differences in comparison with the group of rats with diabetes mellitus.

Administration of enalapril during 14 days to rats with type 2 DM caused decreased amount of TBA in the cerebral cortex and hippocampus 1.1 and 1.5 times respectively. The activity of the antioxidant protection enzymes increased in the CNS of rats with DM. Thus, after enalapril administration SOD activity 3.1 times increased in the cerebral cortex and 1.3 times – in the hippocampus as compared with the indices of the group without treatment. In addition, under enalapril effect catalase activity 1.3 times increased in the cerebral cortex.

 $_{\rm Page} 2735$



Figure 3 Effect of enalapril on the NO-synthase activity in the cytosolic fraction of the brain and hippocampus of rats with type 2 diabetes mellitus ($M \pm m$, n=7)

Notes: * - reliability of differences in comparison with the control group of animals,

** – reliability of differences in comparison with the group of rats with diabetes mellitus.

Table 1 Effect of enalapril on the state of prooxidant-antioxidant system in the cytosolic fraction of the brain and hippocampus of rats with type 2 diabetes mellitus ($M\pm m$, n=7)

Droin structures	Control	Diabatas mallitus	Diabetes mellitus +
Diality structures	Control	Diabetes menitus	enalapril
Cerebral cortex	43.00 ±2.37	79.63±1.56*	72.12±2.26* **
Hippocampus	39.96±3.11	76.92±2.34*	50.04±3.30* **
Cerebral cortex	0.217±0.02	0.115±0.03*	0.359±0.01* **
Hippocampus	0.312±0.01	0.222±0.03*	0.295±0.01**
Cerebral cortex	183.92±9.64	122.31±12.59*	159.36±9.81**
Hippocampus	140.98±12.72	88.99±13.81*	116.48±6.97
	Brain structures Cerebral cortex Hippocampus Cerebral cortex Hippocampus Cerebral cortex Hippocampus	Brain structuresControlCerebral cortex43.00 ±2.37Hippocampus39.96±3.11Cerebral cortex0.217±0.02Hippocampus0.312±0.01Cerebral cortex183.92±9.64Hippocampus140.98±12.72	Brain structuresControlDiabetes mellitusCerebral cortex43.00 ±2.3779.63±1.56*Hippocampus39.96±3.1176.92±2.34*Cerebral cortex0.217±0.020.115±0.03*Hippocampus0.312±0.010.222±0.03*Cerebral cortex183.92±9.64122.31±12.59*Hippocampus140.98±12.7288.99±13.81*

Notes: * - reliability of differences in comparison with the control group of animals,

** – reliability of differences in comparison with the group of rats with diabetes mellitus.

The data in the table 1 are indicative of the fact that NO₂ content in rats with type 2 DM receiving enalapril 2.5 times decreased in the both experimental structures of the brain in comparison with the untreated group. At the same time, NOS activity in the cerebral cortex and hippocampus decreased 1.4 and 1.8 times respectively. It should be noted that the examined indices of NO system after enalapril administration practically reached the control values.

4. DISCUSSION

Thus, the results of our experimental studies evidence the fact of increasing intensity of LPO in the CNS under type 2 DM. The determined LPO increase in the cerebral cortex and hippocampus is a cause of chronic diabetic complications from the side of the CNS such as cognitive disorders and neurodegeneration (Ullah et al., 2016). Moreover, intensification of free radical reactions promotes disregulation of nitrogen oxide system resulting in damage of the vascular endothelial structure and endothelial

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dysfunction. Structural-functional changes of the vascular wall disturb autoregulation of the cerebral circulation and increase probability of ischemic damage of the examined brain structures.

Administration of enalapril as ACEI reduces LPO intensity and stabilizes the indices of NO system in the cerebral cortex and hippocampus, which evidences protector properties available with CNS lesion under condition of type 2 DM. Protective effects of enalapril, first of all, can be associated with improvement of the vascular functional state (dilation prevails over constriction) due to stabilization of NO indices and corresponding decrease of oxidative stress (decreased content of TBA) and activation of the antioxidant protection enzymes. One more possible positive effect of enalapril is a fact of formation of other angiotensins with reduced activity of AlI, namely, A-1-7, AlII, AIV (Jackson et al., 2018). These peptides cause additional stimulation of appropriate receptors and, thus, promote additional vasodilation, anti-proliferation action and tissue regeneration. It is known that the circulatory and local components of RAS play a key role in the processes of neuroinflammation and neurodegeneration, and excessive formation of AlI is considered one of the main causes of neuroinflammation (Sochocka et al., 2017).

Peripheral components of RAS do not have full access to the brain due to the presence of a blood-brain barrier. However, in diseases, its integrity is violated, which allows the components of the RAS to penetrate into the brain (Saraiva et al., 2016). Therefore, it can be assumed that the neuroprotective effect of enalapril as an ACEI is associated with inhibition of the NO system, which can be observed in our studies. As a consequence of reducing the production of reactive oxygen species, inflammatory mediators, slowing down inflammatory and neurodegenerative processes (De Silva & Miller, 2016).

Slowing the progression of neurodegeneration mediated by hyperphosphorylation of tau protein is another possible mechanism of the neuroprotective action of enalapril. Because the blockade of RAS is carried out through PPARy-receptors, which act as a central link in the regulation of insulin and glucose metabolism (Xiaoxue et al., 2019). Therefore, the obtained data are indicative of RAS participation in disorders of the prooxidant-antioxidant system and nitrogen oxide system indices in the CNS with type 2 DM and protector properties of enalapril under conditions of diabetic damage of neurons.

5. CONCLUSION

Under conditions of damaged nervous system induced by type 2 diabetes mellitus the content of products reacting with 2thiobarbituric acid in the cerebral cortex and hippocampus of rats increases, and catalase and superoxide dismutase activity decreases; the content of nitrite-ions and NOS activity increase, which is indicative of intensification of LPO processes and inhibition of the antioxidant protection and nitrogen oxide systems. Under effect of enalapril (14 days) rats with type 2 diabetes mellitus demonstrate the following in both examined structures of the brain: the content of products reacting with 2-thiobarbituric acid decreases, activity of catalase and SOD increases in the cerebral cortex, the content of nitrite-ions in both examined structures of brain decreases, and activity of NOS decreases in the hippocampus only. The obtained results are indicative of a correcting effect of enalapril on the prooxidant-antioxidant balance, and moreover, on the indices of NO system in the cerebral cortex and hippocampus of rats with nervous system damage, which evidences its available neuroprotective properties with central genesis complications due to type 2 DM.

Abbreviations

- DM diabetes mellitus
- CNS central nervous system
- RAS renin-angiotensin systems
- LPO lipid peroxide oxidation
- NO nitrogen oxide
- All angiotensin II
- ACEI angiotensin converting enzyme inhibitors
- Stz streptozotocin
- TBA 2-thiobarbituric acid
- SOD superoxide dismutase
- NOS NO-synthase

Conflict of Interest

The authors declare no conflict of interest or financial support. All authors contributed to the research and/or preparation of the manuscript.

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