MITOCHONDRIAL CEREBRAL DYSFUNCTION IN RATS WITH SCOPOLAMINE-INDUCED NEURODEGENERATION UNDER ENALAPRIL EFFECT


Bukovinian State Medical University, Chernivtsi, Ukraine

Key words: enalapril, scopolamine-induced Alzheimer’s disease, functional state of the mitochondria

Resume. Objective. Neurodestructive diseases are characterized by complex pathobiological cascades in the neuron, which cause disturbances in energy metabolism and the formation of mitochondrial dysfunction. The renin-angiotensin system plays an important role in the physiological functioning of mitochondria, the excessive activity of which increases the risk of neurodegenerative diseases of the brain. Although angiotensin-converting enzyme inhibitors are now considered as means of prevention and treatment of ischemic lesions of the central nervous system, their corrective properties in the development of central neurodegeneration continue to be refined. The objective of our study was investigation of enalapril effect, as an angiotensin-converting enzyme inhibitor, in case of mitochondrial dysfunction of the cerebral cortex and hippocampus of rats under conditions of scopolamine-induced neurodegeneration reproducing development of Alzheimer’s disease in the experiment.

Material and methods. Scopolamine hydrochloride (Sigma, USA) was injected in rats through the peritoneum at a dose of 1 mg/kg for 27 days to simulate Alzheimer’s disease. Starting from the 28th day of the experiment, enalapril was introduced through the peritoneum at a dose of 1 mg/kg, once a day for 14 days.

Results. Under conditions of scopolamine-induced Alzheimer’s disease in the mitochondrial fraction of the cerebral cortex and hippocampus of rats free radical oxidation of lipids and proteins increases, and activity of Krebs cycle enzymes decreases – α-ketoglutarate dehydrogenase and succinate dehydrogenase; light dispersion decreases and a relative rate of mitochondrial swelling increases. After enalapril administration for 14 days to rats with scopolamine-induced Alzheimer’s disease the content of products reacting with 2-thiobarbituric acid and protein oxidation modification decreases in the mitochondrial fraction of the cerebral cortex and hippocampus; in both examined structures, the activity of catalase, α-ketoglutarate dehydrogenase, succinate dehydrogenase increases, and superoxide dismutase – only in the cerebral cortex; a gradual decrease of light dispersion and relative rate of mitochondrial swelling occurs.

Conclusion. Improvement of the antioxidant system state and energy supply of mitochondria, decreased intensity of mitochondrial swelling in the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer’s disease are indicative of the protective properties of enalapril.

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Introduction. Neurodestructive diseases are characterized by complicated pathobiochemical cascades in the neuron, which cause disorders of energy metabolism and the formation of mitochondrial dysfunction. Mitochondria are a primary source of reactive oxygen forms that can regulate calcium balance, intracellular oxidation-reduction processes and influence the cellular signals. Since neurons are limited in their glycolytic abilities, their functional activity depends on mitochondrial energy production more than on other body cells. A significant amount of mitochondria accumulates in the area of synapses. They provide the mechanisms to transmit nerve impulses. Therefore, mitochondrial dysfunction is one of the main pathogenic chains of neurodegenerative processes and Alzheimer’s disease in particular.

According to current scientific data renin-angiotensin system (RAS) plays an essential role in the physiological functioning of mitochondria. Excessive activity of the system increases the risk of neurodegenerative diseases of the brain. Nowadays, the existence of intra-mitochondrial RAS is a well-known fact [1]. Though, in spite of the fact that a considerable amount of results is indicative of the participation of RAS components in central neurodegeneration, the efficacy of pharmacological modulators of RAS remains a subject of scientific investigations [2]. In case of cerebral ischemia, RAS blockers and angiotensin-converting enzyme inhibitors (ACEI), in particular, are known to decrease apoptosis in the hippocampus and considerably improve spatial learning and memory [3]. Although today ACEI are considered as means of prevention and treatment of ischemic injury of the central nervous system, their correcting properties with the development of central neurodegeneration are still being specified [4].

Objective. The objective of our study was to investigate the enalapril effect as an angiotensin-converting enzyme inhibitor in case of mitochondrial dysfunction of the cerebral cortex and hippocampus of rats under conditions of scopolamine-induced neurodegeneration reproducing development of Alzheimer’s disease in the experiment.

Material and methods. The experiments were conducted on nonlinear albino male rats with a bodyweight of 0.18-0.20 kg, kept under standard vivarium conditions with natural day and night changes. Scopolamine hydrochloride (Sigma, USA) was injected into rats through the peritoneum at the dose of 1 mg/kg once a day for 27 days to create Alzheimer’s disease model [5]. On the 28th day of the experiment, a group of rats with Alzheimer’s disease (7 rats) began a course of treatment (14 days) with enalapril introduced through the peritoneum at the dose of 1 mg/kg. A solvent was introduced through the peritoneum into a group of rats with Alzheimer’s disease (7 rats) beginning from the 28th day of the experiment, enalapril was introduced once a day for 14 days.

Results. It was established, that the model of scopolamine-induced Alzheimer’s disease in the experiment was formed in all rats. Mitochondrial fraction was isolated by means of the procedure described elsewhere [6].

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Оригінальні дослідження

differentiation centrifugation method of homogenates of the structures examined. For this purpose, the cerebral cortex and hippocampus were washed with cooled (2-4°C) 0.9 % KCl solution, grinded and homogenized in 10-fold volume of the buffer pH 7.4: sucrose 250 mM, ethylene diamine tetraacetate 1 mM, tris--HCl 10 mM [7].

The state of lipid peroxide oxidation in the mitochondria was evaluated by the levels of active products of thiobarbituric acid (AP TBA); carbonylation of mitochondrial proteins – by the amount of derivatives of 2,4-dinitrophenylhydrazone with the formation of carboxyphenylhydrazone (CPH) and expressed in nmol of carbonyl derivatives per 1 mg of protein [8]. The state of the antioxidant protection system in the mitochondria was evaluated by the activity of superoxide dismutase (SOD) enzymes [EC 1.15.1.1] and catalase [EC 1.11.1.6] [9, 10]. The activity of enzymes α-ketoglutarate dehydrogenase (α-KGDH) [EC 1.2.4.2] and succinate dehydrogenase (SDH) [EC 1.3.5.1] by means of the spectrophotometric method was determined to assess the energy supply of the mitochondria [11, 12]. Swelling of the mitochondria was registered by their ability to extension-contraction and changes in optic density [13, 14]. Changing of E 520 parameters in the incubation medium was used to calculate a relative rate of mitochondrial swelling. This parameter characterizes changes in the permeability of the organelle’s internal membrane (massive swelling and depolarization of mitochondria) resulting from mitochondrial pore formation due to the overloading of cells with calcium ions [15]. The protein content in the mitochondria was determined by the Lowry method [16].

The results of the study were statistically processed using the Student criterion. The Mann-Whitney nonparametric criterion of comparison was also used to confirm the reliability of the conclusions. It demonstrated similar results of calculations made applying the Student criterion concerning the p value. Differences were considered statistically valuable with p≤0.05.

**Results.** The results of our investigations showed (Table) that scopolamine-induced Alzheimer’s disease is characterized by disorders of the prooxidant-antioxidant balance in the mitochondria of the cerebral cortex and hippocampus. 70.3 % increase in AP TBA content in the cerebral cortex and 100.9 % increase in the hippocampus were determined in comparison with the parameters of the control group. CPH content in the mitochondria of both examined structures was 46.5 % increased. The data obtained were indicative of the intensification of free radical oxidation processes of lipids and proteins that promoted injury of biological membranes. At the same time, functional disorders of the antioxidant protection enzymatic systems were found by the indices of SOD and catalase activity.

Thus, SOD activity in the cerebral cortex 27.9 % decreased, and only the tendency to its activity decrease was found in the hippocampus. Catalase activity in both examined structures decreased: 25.4 % in the cerebral cortex and 43.6 % in the hippocampus. The data obtained are first of all indicative of cerebral cortex injury in rats with simulated Alzheimer’s disease. Second, they are indicative of greater injury of the hippocampus as an important chain in the development of neurodestructive pathology.

Moreover, on the basis of the investigations conducted decrease in dehydrogenase activity was found in the group of rats with simulated pathology, which determines the efficacy of the mitochondrial energy supply of the brain (Fig. 1, 2). In fact, α-KGDH activity (NAD+–dependent enzyme) 35.4 % decreased in the cerebral cortex and 43 % – in the hippocampus. At the same time, the activity of FAD–dependent SDH in both examined structures was 63.6 and 54.8 % decreased, respectively. According to the literary data, disorders of oxidation processes and excessive formation of free radicals at the early stages of the development of Alzheimer’s disease can result in the irreversible opening of a mitochondrial pore, promoting the apoptosis process [17].

Further investigations showed that after enalapril administration the content of AP TBA 23.4 % decreased in the cerebral cortex, and 33.9 % - in the hippocampus. At the same time, the content of CPH in both structures 29.6 % decreased in the cerebral cortex and 31.1 % – in the hippocampus. The results obtained are indicative of inhibition of lipid and protein peroxidation processes.

A positive effect of enalapril on the activity of antioxidant protection enzymes should be mentioned.

**Table**

<table>
<thead>
<tr>
<th>Indices</th>
<th>Examined cerebral structures</th>
<th>Control</th>
<th>Alzheimer’s disease</th>
<th>Alzheimer’s disease + enalapril</th>
</tr>
</thead>
<tbody>
<tr>
<td>The content of AP TBA, nmol/mg of protein</td>
<td>Cortex</td>
<td>12.78±1.251</td>
<td>21.77±1.502*</td>
<td>16.71±0.872**</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>11.63±0.652</td>
<td>23.34±1.301*</td>
<td>15.39±0.930**</td>
</tr>
<tr>
<td>The content CPH, nmol/mg of protein</td>
<td>Cortex</td>
<td>24.67±1.391</td>
<td>36.17±2.623*</td>
<td>25.54±1.182**</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>18.29±1.102</td>
<td>26.68±1.209*</td>
<td>18.42±1.011**</td>
</tr>
<tr>
<td>The activity of SOD, units /mg of protein</td>
<td>Cortex</td>
<td>0.428±0.0271</td>
<td>0.307±0.0173*</td>
<td>0.497±0.0251**</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>0.379±0.0449</td>
<td>0.257±0.0541</td>
<td>0.331±0.0462</td>
</tr>
<tr>
<td>The activity of catalase, mcnmol H2O2/min of mg of protein</td>
<td>Cortex</td>
<td>175.87±10.576</td>
<td>131.17±10.018*</td>
<td>184.57±15.898**</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>170.21±10.990</td>
<td>92.56±15.159*</td>
<td>157.29±10.547**</td>
</tr>
</tbody>
</table>

**Note.** * – reliability of differences compared with the control group of rats; ** – reliability differences compared with the group of rats with Alzheimer’s disease.
Fig. 1. Enalapril effect on the activity of α-ketoglutarate dehydrogenase in the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer’s disease (M±m, n=7). Note. * – reliability of differences compared with the control group of rats, ** – reliability of differences compared with the group of rats with Alzheimer’s disease.

Fig. 2. Enalapril effect on the activity of succinate dehydrogenase in the mitochondria of the cerebral cortex and hippocampus of rats with scopolamine-induced Alzheimer’s disease (M±m, n=7). Note. * – reliability of differences compared with the control group of rats; ** – reliability of differences compared with the group of rats with Alzheimer’s disease.

Thus, activity of SOD and catalase in the cerebral cortex and hippocampus increased: 29.7% and 40.7%, respectively. In the hippocampus 69.9% increase of catalase was found, which confirms the primary injury of hippocampus with scopolamine-induced Alzheimer’s disease. At the same time, increased activity of Krebs cycle enzymes was found: α-KGDH and SDH activity 24.9 and 100% increased in the cerebral cortex and in the hippocampus – 45.9 and 78.8%, respectively.

As it was mentioned above, synapses are the first “indicator” of disorders in case of Alzheimer’s disease, since they require considerable energy supply. Therefore, we got interested in functional changes in mitochondria as energy producing structures in the cerebral cortex and hippocampus under conditions of neurodegeneration development and assess enalapril effect on their functional state.

Fig. 3a, b presents the dynamics of changes in the mitochondrial swelling intensity of the examined brain structures. In rats of the control group, after 60-minute incubation of mitochondrial suspension of the cerebral cortex and hippocampus, the level of light dispersion 1.1 times decreased. In rats with Alzheimer’s disease, light dispersion of mitochondrial suspension 60 minutes later 1.2 times decreased in the cerebral cortex and 1.3 times – in the hippocampus. Enalapril administration for 14 days to rats with Alzheimer’s disease led to a gradual decrease in light dispersion in the cerebral cortex and the hippocampus – 1.0 and 1.1 times, respectively.

Further analysis of investigations showed that a relative rate of mitochondrial swelling intensity which is measured in UN/min/mg of protein in rats with scopolamine-induced Alzheimer’s disease increase in comparison with the control group in the cerebral cortex from 1.6±0.014 to 1.9±0.008 and in the hippocampus – from 1.8±0.009 to...
Discussion. Thus, in rats with scopolamine-induced Alzheimer’s disease the pore of non-specific permeability of the mitochondria is activated, as it is a regulator of ion and protein transport. Respectively, the intracellular Ca²⁺ pool increases and imbalance between the cytosolic and mitochondrial ion levels occurs. These processes induce disorders of prooxidant-antioxidant balance in the mitochondria.

Under conditions of enalapril administration in rats with Alzheimer’s disease the activity of the antioxidant system enzymes and Krebs cycle increased, the content of products of lipid and protein peroxidation and intensity of mitochondrial swelling decreased. Inhibition of oxygen reactive forms production is a probable mechanism of the agent action, which is stimulated by angiotensin II. It results in the decrease of AP TBA and CPH content and increase of antioxidant enzymes activity both in the cerebral cortex and hippocampus. The above processes inhibit development of mitochondrial dysfunction due to the blockade of NADH-oxidase of the endothelial cells and inhibition of peroxinitrite formation, which promotes improvement of the cerebral circulation and inhibits ischemic-hypoxic effects on the central nervous system [18].

One of the important mechanisms in enalapril action is the fact that blockade of angiotensin II stimulates K⁺-mediated release of acetylcholine – one of the primary neurotransmitters of the cholinergic system. Clinical signs of Alzheimer’s disease are associated with the loss of cholinergic innervation in the cerebral cortex [19], which is confirmed by clinically evidenced efficacy of

**Fig. 3a. Intensity of mitochondrial swelling in the cerebral cortex of rats with scopolamine-induced Alzheimer’s disease after enalapril administration during 14 days in the dose of 1 mg/kg.**

*Note. The results are represented as Me[min-max], n=7, p≤0.05*

**Fig. 3b. Intensity of mitochondrial swelling in the hippocampus of rats with scopolamine-induced Alzheimer’s disease after enalapril administration during 14 days in the dose of 1 mg/kg.**

*Note. The results are represented as Me[min-max], n=7, p≤0.05*
anticholinesterase preparations of a central action [20].

Therefore, the results obtained are indicative of the fact that enalapril under conditions of scopalamine-induced Alzheimer’s disease in rats possesses a protective effect, which is characterized by improvement of a functional state and parameters of the mitochondrial energy metabolism, increased activity of the antioxidant system in the cerebral cortex and hippocampus.

**Conclusion.** Under conditions of scopalamine-induced Alzheimer’s disease in the mitochondrial fraction of the cerebral cortex and hippocampus of rats, free radical oxidation of lipids and proteins increases, and activity of Krebs cycle enzymes decreases – α-ketoglutarate dehydrogenase and succinate dehydrogenase: light dispersion decreases and a relative rate of mitochondrial swelling increases.

After enalapril administration for 14 days to rats with scopalamine-induced Alzheimer’s disease, the content of products reacting with 2-thiobarbituric acid and protein oxidation modification decreases in the mitochondrial fraction of the cerebral cortex and hippocampus; in both examined structures the activity of catalase, α-ketoglutarate dehydrogenase, succinate dehydrogenase increases, and superoxide dismutase – only in the cerebral cortex; a gradual decrease of light dispersion and relative rate of mitochondrial swelling occurs.

Improvement of the antioxidant system state and energy supply of mitochondria, decreased intensity of mitochondrial swelling in the cerebral cortex and hippocampus of rats with scopalamine-induced Alzheimer’s disease are indicative of protective properties of enalapril.

**Prospects for further research.** It is planned to study the effect of enalapril on the morphological state of the cerebral cortex and hippocampus under the conditions of experimental neurodegenerations of different genesis.

**References**


Оригінальні дослідження

University, Chernivtsi, Ukraine. ORCID: https://orcid.org/0000-0001-8582-6685. E-mail: filipec.natalja@bsmu.edu.ua.

Кмет Т.І. – Doctor of Medical Sciences, Professor of the Department of Hygiene and Ecology, Bukovinian State Medical University, Chernivtsi, Ukraine. ORCID: https://orcid.org/0000-0002-2850-9111. E-mail: kmet.taras@bsmu.edu.ua.

Andriychuk N. Y. – PhD, Associate Professor of the Department of Hygiene and Ecology, Bukovinian State Medical University, Chernivtsi, Ukraine. E-mail: nadin11ua@gmail.com.

Тимкул Д. М. – Assistant Professor of the Department of Hygiene and Ecology, Bukovinian State Medical University, Chernivtsi, Ukraine. E-mail: dianatimkul@gmail.com.

Відомості про авторів

Кметь О. Г. – канд. мед. наук, доц. кафедри фармакології Буковинського державного медичного університету, м. Чернівці, Україна. ORCID: https://orcid.org/0000-0003-0336-1103. E-mail: kmet.olga@bsmu.edu.ua.

Філіпець Н. Д. – д-р мед. наук, проф. кафедри фармакології Буковинського державного медичного університету, м. Чернівці, Україна. ORCID: https://orcid.org/0000-0001-8582-6685. E-mail: filipec.natalja@bsmu.edu.ua.

Кметь Т.І. – д-р мед. наук, проф. кафедри гігієни та екології Буковинського державного медичного університету, м. Чернівці, Україна. ORCID: https://orcid.org/0000-0002-2850-9111. E-mail: kmet.taras@bsmu.edu.ua.

Андрійчук Н. Й. – канд. мед. наук, доц. кафедри гігієни та екології Буковинського державного медичного університету, м. Чернівці, Україна. E-mail: nadin11ua@gmail.com.

Тимкул Д. М. – асистент кафедри гігієни та екології Буковинського державного медичного університету, м. Чернівці, Україна. E-mail: dianatimkul@gmail.com.

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