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RESEARCH OF ENERGOTROPIC PROPERTIES OF 3-BENZYLXANTHINE DERIVATIVE – PROSPECTIVE NEUROPROTECTOR

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ABSTRACT

Background: Acute cerebrovascular accident (CVA) represent a leading cause of mortality in developed countries. Ischemic stroke results in pronounced structural changes in cerebral tissue, which in turn play a central role in perturbation of cognitive and associative functions of the brain. An outcome of hypoxia is the mitochondrial dysfunction, which manifests in sequential phase changes in mitochondrial enzymatic complexes, and leads to inhibition of aerobic energy synthesis, energy-dependent functions, and cell metabolism. Aims and objectives: The present study was carried out to investigate the energotropic properties of potential neuroprotector – xanthine derivative Ale-15 in conditions of experimental ischemia. Materials and Methods: Experimental part was done on white Wistar rats. For assessment of energotropic propeties of Ale-15 compound we used a model of global incomplete cerebral ischemia. The state of energy metabolism was determined by the level of the most important intermediates – ATP, ADP, AMP and by following indices of energy metabolism: the energy charge, energy potential, coefficient of comparison, index of phosphorylation and thermodynamic respiration control. Results: Xanthine derivative Ale-15 stabilized the energy state of the neurons of animals with CVA, as evidenced by increasing levels of ATP and ADP on the background of decreasing of AMP level. Analysis of additional energy parameters showed, that the compound increased the degree of filling ATP-ADP-AMP system by macroergic bonds, activated the direct reaction of ATP synthesis, corrected the imbalance of ratio of macroergic phosphates and intensity of phosphorylation of adenyl-nucleotide system in generally. All these allowed suggesting, that studied compound Ale-15 possessed energotropic effect in conditions of cerebral ischemia. Conclusion: The present study showed the energotropic properties of xanthine derivative Ale-15 in condition of ischemic stroke, which exceeded the effect of reference drug - Mexidol.

Key words: Xanthine, Energotropic properties, Neuroprotection, Ischemic stroke.

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INTRODUCTION

Acute cerebrovascular accident (CVA) represents a leading cause of mortality in developed countries. They take the third place in prevalence in Europe and countries of the American continent, next only to cardiovascular pathologies and malignant tumors [1]. Ischemic stroke results in pronounced structural changes in cerebral tissue, which in turn play a central role in perturbation of cognitive and associative functions of the brain. These changes are induced by suppression of bioenergetic processes, development of glutamate excitotoxicity, and hyperproduction of reactive oxygen species (ROS) in cerebral tissues during stroke [2,3].

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In a state of acute cerebral hypoxia (on the background of decreased activity of the antioxidant neuroprotective mechanisms) accumulation of ROS leads to excessive formation of NO-radical. The latter inhibits activity of the mitochondrial electron-transport chain and TCA/Krebs cycle enzymes, a condition that subsequently results in a decrease in ATP synthesis, depletion of ATP depots, and neuronal death via necrosis or apoptosis. NO-radical reacts with the superoxide anion, a compound synthesized in conditions of oxygen deficit, resulting in formation of highly toxic peroxynitrite radical. Via the chain-reaction mechanism, formed free radical substrates accelerate neuronal cell death through inhibition of activity of iron- and sulphur-containing enzymes of TCA/Krebs cycle and the electron transport chain [4,5]. An outcome of hypoxia is the mitochondrial dysfunction, which manifests in sequential phase changes in mitochondrial enzymatic complexes, and leads to inhibition of aerobic energy synthesis, energy-dependent functions, and cell metabolism [4,6].

Substituted xanthine derivatives are an important class of biologically active compounds that exhibited various pharmacological properties [7,8]. Earlier we mentioned about the Ale-15 compound – (morpholin-4-ium 3-benzylxanthinyl-8-methylthioacetate), that shows high antioxidant qualities *in vitro* [9] and neuroprotective properties *in vivo* [10].

The goal of this research was to study energotropic effect of Ale-15 compound in conditions of bilateral common carotid arteries ligation and in comparison with pharmocological standard – Mexidol [11,12].

MATERIALS AND METHODS

Animals

Experimental part was done on white Wistar rats of both sexes of 220-260 g weight. All animals were on standard food ration of vivarium, with natural alteration of day and night. Rats were received from nursery of Institute of Pharmacology and Toxicology of Ukraine. All experimental procedures and operative interventions were done in accordance with WMA Statement on Animal Use in Biomedical Research.

Stroke model

For assessment of activity of compound we used a model of global incomplete cerebral ischemia, which is the most adequate in terms of clinical implications of ischemic stroke [13]. This model was reproduced by bilateral common carotid arteries ligation that was performed under Nembutal anesthesia (40 mg/ kg), with implication of surgical approach by means of separation of carotid arteries and single-step silk deligation [13]. We took 1/20 LD_{50} as the relatively curative dose [10]. The compound Ale-15 under research was injected once a day during the whole experiment at a dose of 50 mg/kg intragastrically with the help of metal catheter; Mexidol was injected according to the same schedule at a dose of 250 mg/kg intragastrically. The intact group was presented by pseudo-operated animals, which underwent the separation of carotid arteries under Nembutal anesthesia (40 mg/ kg), with implication of surgical approach without deligation.

Biochemical analysis

Materials

For investigation of long-term results of pharmacological correction we took brain from experimental animals on the fourth day after the operation. We used cerebral cortex frontal lobes for biochemical investigation. For biochemical investigation cerebral tissues were homogenized in cold in salt isotonic solution (0.15 M KCl) at the temperature of $+4^{o}C$ with the help of glass homogenizer, in ratio tissue – salt solution 1:10. After that, we separated cytosolic fraction (15000 g) by means of differential centrifugation. Extract, deprived of proteins, was obtained precise weight of homogenate of cerebral tissue in 0.6 M solution of $HClO_4$ with further neutralization with 5.0 M solution of potassium carbonate [14].

Energy state of the cell

Energy state of the cell was determined by the level of the most important intermediates – ATP, ADP, AMP. Adenylic nucleotides were determined by thin layer chromatography [14]. For in-depth analysis of the energy supply of neurons in condition of CVA, we calculated the following indices of energy metabolism: the energy charge (EC), energy potential (EP), coefficient of comparison (CC), index of phosphorylation (IP) and thermodynamic respiration control (TRC) [15].

Statistical analysis

The statistic data processing was carried out with the help of software for statistic data processing STATISTICA® for Windows 6.0 (StatSoft Inc. AXXR712D833214FAN5) [16]. The data is presented by sample mean ± standard error of the mean. The control of distribution normalcy was done in accordance with Shapiro-Wilk test. The fidelity of differences between experimental groups was estimated with the help of Student's t-test and of Whitney-Mann test.

RESULTS

Biochemical investigations showed that double-sided common carotid arteries ligation led to the imbalance of macroergic phosphates in the brain of rats with CVA (Table 1). So, on the 4th day of experiment the level of AMP increased on 133.6 % against decreasing of the ADP (on 54.4 %) and ATP (on 46.6 %) levels, that could be explained by intensified decay of ATP during ischemic damage.

Table 1. Influence of Ale-15 on the content of adenylic nucleotides in brain of animals on the 4th day after CVA

Animals Group	ATP µM/g of tissue	ADP µM/g of tissue	AMP µM/g of tissue
Sham surgery animals	2.015±0.098	0.529±0.071	0.116±0.011
Animals with CVA	1.076±0.047	0.241±0.008	0.271±0.007
Animals with CVA+Ale-15	1.710±0.103*	0.464±0.028*	0.119±0.009*
Animals with CVA+Mexidol	1.333±0.086*	0.448±0.053*	0.148±0.013*

Remark: * - $p \leq 0.05$ in relation to control

In-depth analysis of the energy supply of neurons showed decreasing of intensity and efficiency of phosphorylation of adenyl-nucleotide system (Table 2).

Table 2. Influence of Ale-15 on the indices of energy supply of neurons in brains of rats on the 4th day after CVA

Animals group	EC	EP	CC	IP	TRC
Sham surgery animals	0.858 ± 0.008	4.026 ± 0.434	4.252 ± 0.449	3.269 ± 0.309	4.518 ± 0.331
Animals with CVA	0.752 ± 0.01	4.481 ± 0.294	5.606 ± 0.297	2.109 ± 0.137	0.890 ± 0.016
Animals with CVA+Ale-15	0.845 ± 0.006*	3.741 ± 0.324*	4.005 ± 0.346*	2.951 ± 0.218*	4.040 ± 0.515*
Animals with CVA+Mexidol	0.806 ± 0.015*	3.153 ± 0.418*	3.497 ± 0.440*	2.333 ± 0.285*	3.061 ± 0.334*

Remark: * - $p \leq 0.05$ in relation to control

DISCUSSION

Injection of Mexidol in the acute stage of ischemic stroke led to the partial normalization of content of adenyl-nucleotides, such as increasing ATP content on 23.7 % and decreasing AMP level on 45.4 %. Administration of xanthine derivative Ale-15 increased the synthesis of ATP up to the level of intact group, which exceeded effect of Mexidol (Table 1).

In-depth analysis of the energy supply of neurons showed that index of the energy charge decreased on 12.3 % in control group in comparison with sham surgery animals, which reflected a significant reduction in the degree of filling of ATP-ADP-AMP system by macroergic bonds. Treatment of animals by Ale-15 compound led to increasing of EC up to intact level. In action of Mexidol was marked similar but less pronounced effect.

Studying of energy potential showed it increasing in control group in the acute period of experimental ischemia on 11.3 % that indicated about activation of mitochondrial electron transport chain and correlated with changes in content of adenyl-nucleotide pool [17]. Experimental therapy by antioxidants (Mexidol and xanthine derivative Ale-15) led to correction of mitochondrial disorders and to decreasing of EP down to the intact level and below. These changes demonstrated stabilizing effect of studied compounds on tissue respiration.

Similar changes were determined during analysis of coefficient of comparison (CC), which describes ratio of ATP and AMP summary to ADP. CC increased in control group on 31.8 % that was caused by increasing of AMP level and predominance of direct reaction of ADP transformation under indirect reaction.

Injections of Ale-15 compound and Mexidol led to decreasing of CC level (on 28.5 % and 37.6 % respectively) in comparison with control group. These data confirmed positive influence of studied compounds on stabilizing of tissue respiration and correction of energy imbalance.

Analysis of index of phosphorylation in the brain of experimental animals with CVA allowed establishing violation ratio between ATP and ADP-AMP pool in control group (decreasing IP on 35.4 %). In the brain tissue of animals, that there were treated by Ale-15 compound, IP were higher than in control group. That indicated on correction of imbalance of ratio of some macroergic phosphates in conditions of ischemic stroke. It should be noted, that stabilizing influence of xanthine derivative on IP marker exceeded effect of Mexidol.

Marker of thermodynamic respiration control, which displayed dependence of activity of mitochondrial respiratory chain from concentration of some components of adenyl-nucleotide system and intensity of phosphorylation in generally, were decreased in brain of rats with CVA in more than 5 times. On the background of Mexidol administration, there were observed a significant increasing of TRC (on 244 %), but this index still was lower in comparison with intact group (in 1.5 times). The stabilizing effect of xanthine derivatives on TRC marker exceeded the effect of Mexidol. Injection of Ale-15 compound increased TRC marker up to 89 % from intact group level, that in 1.3 times higher than effect of referent drug.

Thus, the analysis of obtained results showed that xanthine derivative Ale-15 stabilized the energy state of the neurons of animals with CVA, as evidenced by increasing levels of ATP and ADP on the background of decreasing of AMP level. Analysis of additional energy parameters showed, that the compound increased the degree of filling ATP-ADP-AMP system by macroergic bonds, activated the direct reaction of ATP synthesis, corrected the imbalance of ratio of macroergic phosphates and intensity of phosphorylation of adenyl-nucleotide system in generally.

All these allowed suggesting, that studied compound Ale-15 possessed energotropic effect in conditions of cerebral ischemia. Comparative analysis of activity of Mexidol and Ale-15 compound revealed that more effective correction of ATP, ADP and AMP levels set in group of xanthine derivative, which indicated on pronounced energotropic action.

CONCLUSION

The present study showed the energotropic properties of xanthine derivative Ale-15 in condition of bilateral ligation of the common carotid artery (ischemic stroke), which exceeded the effect of reference drug - Mexidol. Obtained results are experimental basis for further search neuroprotectors with energotropic effect among substituted xanthine derivatives.

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