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Correction of mitochondrial dysfunction with trimethoxy-substituted monocarbonyl curcumin analogues in experimental Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disease that is a terminal form of dementia with an alarming spread rate. The treatment of AD usually involves symptomatic therapy, but the research field for new medicines to correct AD focus on the pathogenetic keys of the disease, i.e., a mitochondrial dysfunction.

The aim of the work was to evaluate the effect of trimethoxy-substituted monocarbonyl curcumin analogues on changes in the mitochondrial function of the hippocampus in AD rats.

Materials and methods. AD was modeled in female Wistar rats by the injection of β-amyloid aggregates 1-42 into the CA1 part of the hippocampus. The tested compounds AZBAX4 and AZBAX6 at a dose of 20 mg/kg each, as well as the reference donepezil at a dose of 50 mg/kg, were administered orally for 30 days after the surgery. After the specified time had passed, the changes in the cellular respiration, a citrate synthase activity, cytochrome-c-oxidase, succinate dehydrogenase, and adenosine triphosphate (ATP) concentrations were evaluated in the mitochondrial fraction of the rat hippocampus.

Results. During the study, it was shown that the use of AZBAX4 and AZBAX6 compounds contributed to an increase in the intensity of aerobic metabolism by 83.9 (p <0.05) and 35.9% (p <0.05), respectively, while reducing the activity of anaerobic one by 27.7 (p <0.05) and 20.6% (p <0.05), respectively. Against the background of the tested compounds AZBAX4 and AZBAX6 administration, there was also a significant increase in the activity of citrate synthase, succinate dehydrogenase and cytochrome-c-oxidase, as well as the level of ATP in the hippocampal tissue by 112.8 (p <0.05) and 117.1% (p <0.05), respectively. The use of donepezil led to a significant increase in the intensity of aerobic reactions – by 24.0% (p <0.05), a citrate synthase activity– by 80.0% (p <0.05) and the ATP concentration – by 68.5% (p <0.05). Against the background of the use of the analyzed substances, a decrease in the apoptosis-inducing factor and mitochondrial hydrogen peroxide is also worth noting.

Conclusion. Based on the obtained data, it can be assumed that the use of AZBAX4 and AZBAX6 compounds contributes to an increase in the functional activity of the mitochondria of hippocampal cells of AD rats, while surpassing the reference donepezil. It is perspective to continue a further study of AZBAX4 and AZBAX6 compounds as possible medicines of a pathogenetic correction of AD.

Keywords: Alzheimer's disease; mitochondrial dysfunction; curcumin analogues; neuroprotection

Abbreviations: AD – Alzheimer's disease; Aß – amyloid β; SO – sham-operated animals; NC – negative control; SDH – succinate dehydrogenase; CoX – cytochrome-c-oxidase; CS – citrate synthase; ATP – adenosine triphosphate; TOMM – transporter of the outer mitochondrial membrane; AßPP – precursor protein β-amyloid; OCR – oxygen consumption rate, AIF – apoptosis-inducing factor; MitoH_2O_2 – mitochondrial hydrogen peroxide.

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Коррекция митохондриальной дисфункции триметокси-замещенными монокарбонильными аналогами куркумина в условиях экспериментальной болезни Альцгеймера

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Болезнь Альцгеймера (БА) – нейродегенеративное заболевание, представляющее собой терминальную форму деменции с угрожающими темпами распространения. Лечение БА подразумевает, как правило, симптоматическую терапию, однако области поиска новых средств для коррекции БА сосредотачиваются на патогенетических особенностях заболевания, например, митохондриальной дисфункции.

Цель. Изучить влияние триметокси-замещенных монокарбонильных аналогов куркумина на изменение митохондриальной функции гиппокампа у крыс с экспериментальной БА.

Материалы и методы. БА моделировали у крыс-самок линии Wistar путем введения агрегатов β-амилоида 1-42 в СА1 часть гиппокампа. Анализируемые соединения с шифрами AZBAX4 и AZBAX6 в дозе 20 мг/кг каждое, а также препарат сравнения донепезил в дозе 50 мг/кг вводили перорально на протяжении 30 дней с момента проведения оперативного вмешательства. По истечении указанного времени в митохондриальной фракции гиппокампа крыс оценивали изменение клеточного дыхания, активности цитратсинтазы, цитохром-с-оксидазы, сукцинатдегидрогеназы и концентрации аденозинтрифосфата (АТФ), апоптоз-индуцирующего фактора и митохондриального пероксида водорода.

Результаты. В ходе исследования было показано, что применение соединений AZBAX4 и AZBAX6 способствовало повышению интенсивности аэробного метаболизма на 83,9 (p <0,05) и 35,9% (p <0,05) соответственно, при снижении активности анаэробного на 27,7 (p <0,05) и 20,6% (p <0,05) соответственно. Также на фоне введения анализируемых соединений AZBAX4 и AZBAX6 отмечено достоверное повышение активности цитратсинтазы, сукцинатдегидрогеназы и цитохром-с-оксидазы, а также уровня АТФ в ткани гиппокампа на 112,8 (p <0,05) и 117,1% (p <0,05) соответственно. Применение донепезила приводило к статистически значимому увеличению интенсивности аэробных реакций – на 24,0% (p <0,05), активности цитратсинтазы – на 80,0% (p <0,05) и концентрации АТФ – на 68,5% (p <0,05). Также стоит отметить уменьшение апоптоз-индуцирующего фактора и митохондриального пероксида водорода на фоне применения анализируемых веществ.

Заключение. На основании полученных данных можно предполагать, что применение соединений AZBAX4 и AZBAX6 способствовало повышению функциональной активности митохондрий клеток гиппокампа крыс с БА, превосходя при этом референт донепезил. Целесообразно продолжить дальнейшее изучение соединений AZBAX4 и AZBAX6 как возможных средств патогенетической коррекции БА.

Ключевые слова: болезнь Альцгеймера; митохондриальная дисфункция; аналоги куркумина; нейропротекция

Список сокращений: БА – болезнь Альцгеймера; Аβ – амилоид β; ГМ – головной мозг; ЛО – ложнооперированные животные; НК – негативный контроль; СДГ – сукцинатдегидрогеназа; СоХ – цитохром-с-оксидаза; ЦС – цитратсинтаза; АТФ – аденозинтрифосфат; TOMM – транспортер внешней митохондриальной мембраны; АβРР – белок предшественник β-амилоида, OCR – потребление кислорода; АИФ – апоптоз-индуцирующий фактор; MitoH_2O_2 – митохондриальный пероксид водорода.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease; its main clinical symptom is progressive cognitive deficits. AD ranks 6 among the leading causes of death, with the AD mortality rate increasing by 145% between 2000 and 2019 [1], while deaths from cardiovascular disease, diabetes mellitus, and a HIV infection declined over time. In addition to the high epidemiological component, AD has a significant impact on the health care economy. So, in 2022, 339.5 billion of US dollars were spent on the care of patients suffering from AD [2].

On the pathophysiology level, AD is characterized by a loss of neurons, a synaptic dysfunction, and the formation of hyperphosphorylated tau protein aggregates, as well as pathological inclusions of beta-

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amyloid [3]. Important elements of the pathogenesis of AD are: an oxidative stress, inflammatory reactions, an excitotoxicity, an imbalance of steroid hormones (estrogens, androgens, and corticosteroids) and the endocanabinoid system [4]. However, despite the accumulated scientific and practical experience in studying the pathophysiology and therapeutic strategies of AD, many problems remain unresolved. One of them is the matter of the beta-amyloid neurotoxicity mechanisms and its mediated loss of cognitive functions, which is the main drawback of the amyloid theory of AD development. In order to interpret the existing amyloid hypothesis of AD, several concepts have been proposed, the leading one being the «mitochondrial cascade» theory [5]. The main provisions of the «mitochondrial cascade» hypothesis imply the provision about the most pathological changes in the brain tissue (BT) in AD basing on the mitochondrial dysfunction (MD).

Mitochondria are primarily known as organelles that perform energy-producing, redox- and apoptosis-regulating functions. In addition, mitochondria are necessary for the synthesis of neuronal iron-sulfur protein and haeme, as well as the operation of neuronal reuptake transporters. Mitochondria provide an important buffer mechanism for regulating calcium concentrations. Given the fact that BT neurons are the cells that are extremely dependent on the energy deficiency, contain high concentrations of calcium, and are sensitive to the oxidative damage, a mitochondrial dysfunction will significantly affect their survival [6]. To date, a large number of studies have demonstrated that a mitochondrial dysfunction invariably precedes the clinical manifestation of AD, and under the data of positron emission tomography using fluoro-2-deoxyglucose, the most negative changes occur in the hippocampus and cerebral cortex [7].

Since MD is the main link between all suspected parts of the pathogenesis of AD, targeting the cell mitochondria may be a new approach to treating AD. At the same time, substances that correct disturbed processes in mitochondria seem to compensate for the energy deficit and eliminate oxidative stress, which leads to the restoration of various metabolic pathways, including the Krebs cycle, beta-oxidation of fatty acids, oxidative phosphorylation, haeme biosynthesis, and glycolysis [7].

According to Bhatia S. et al., 14 clinical trials aimed at studying the possibility of correcting MD in AD were registered in 2022. At the same time, most studies indicate that the most pronounced effect is provided by antioxidant substances: MitoQ, epigallocatechin gallate, pramipexole, resveratrol, Centella asiatica extract, a combination of omega-3 polyunsaturated fatty acids with alpha-lipoic acid [9]. It has also been shown that some cinnamic acid derivatives [10] and glycetein [11] can reduce the manifestations of MD neurons in AD. However, the search for new compounds of MD correction remains relevant. Chainoglou E. et al. indicates that bis-symmetric compounds with the structure similar to curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) – curcuminoids may be promising objects for studying as AD therapy agents in the context of restoring a mitochondrial function of neurons [12].

THE AIM of the work was to evaluate the effect of trimethoxy-substituted monocarbonyl curcuminoids on changes in hippocampal mitochondrial function in rats with experimental AD.

MATERIALS AND METHODS

Experimental animals

The study was performed on 50 adult female Wistar rats with a body weight 200–220 g, obtained from the laboratory animal nursery "Rappolovo" (Russia). During the experiment, the animals were kept in the standard conditions of the vivarium of Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University (PMPI) in polypropylene boxes by 5 individuals in each. The admission of rats to complete extruded food and water was not restricted. The litter material (a granulated hardwood fraction) was changed at least once every 3 days. The conditions of keeping excluded the animal stress: the ambient temperature of 22±2°C, the relative humidity of 55–65% and a 12-hour daily cycle. All manipulations were performed under chloral hydrate anesthesia (an intraperitoneal injection of chloral hydrate (PanReac Applichem, Spain) at a dose of 350 mg/kg). The euthanasia of the animals was performed after the anesthesia and removal of biomaterials by a cervical dislocation. The work concept was approved by the Local Ethics Committee of PMPI (Protocol No. 8 dated Jul 7, 2023) and was in the line with the provisions of Directives of EU 2010/63 and the principles of ARRIVE 2.0 [13].

Experimental model of Alzheimer's disease

AD of a sporadic phenotype was modeled in rats by injecting fragments of ß-amyloid 1-42 ($Aß_{1-42}$) into the CA1 segment of the hippocampus (the stereotactic coordinates were as follows: anteroposterior – 3.8 mm, medial-lateral – 2 mm, dorsal-ventral – 2.6 mm from the bregma). Aggregates $Aß_{1-42}$ (Sigma-Aldrich, Germany) were prepared by dissolving $Aß_{1-42}$ protein fragments in a mixture of dimethyl sulfoxide (Vekton, Russia) and a phosphate-salt buffer solution (pH=7.4) in a ratio of

1:10 at the temperature of 4°C. The resulting solution was incubated for 3 days with continuous stirring. Aß$_{1-42}$ aggregates were administered to the animals anesthetized with chloral hydrate, for which the parietal area was scalped and a trepanation hole was made. Then, using a microdoser with a G30 needle, Aß$_{1-42}$ aggregates were introduced in a final concentration of 1 mmol/l in a volume of 5 µl. The needle remained at the injection site for 5 min, after which it was removed. The wound was sutured and treated with a 10% povidone-iodine solution (Betadine Egis Pharmaceutical Plant, Russia) [14].

Analyzed compounds

The test compounds (1E, 4E)-1,5-bis(3,4,5-trimethoxyphenyl)penta-1,4-diene-3-one (cipher AZBAX4) and (1E, 4E)-1,5-bis(2,4,6-trimethoxyphenyl) penta-1,4-diene-3-one (cipher AZBAX6) were obtained at the Department of Organic Chemistry of PMPI. The structure of the analyzed substances was identified by IR, UV, and NMR spectroscopy [15]. The studied compounds were administered orally at a dose of 20 mg/kg in the form of a fine-dispersed suspension prepared *ex tempore* on the basis of a phosphate-salt buffer solution with pH=7.4 [16]. Donepezil (Alzepil®, Egis Pharmaceutical Plant, Russia) was used as a reference drug at a dose of 50 mg/kg, initially as a suspension [17]. The duration of the administration of the studied compoundss and the reference agent was similar and amounted to 30 days from the moment of the AD modeling.

Experimental groups

All the animals were randomized by a body weight (no more than a 10% deviation in the group and between the groups) into 5 equal groups of 10 individuals in each: sham-operated animals (SO); negative control (NC); a group of the animals receiving the reference drug (donepezil); a group of the animals receiving the analyzed compound under the cipher AZBAX4; a group of the animals that received the analyzed compound under the cipher AZBAX6. The rats of all the groups, with the exception of SO, were modeled AD according to the method described above. The SO group of rats was consistently treated with the manipulations similar to those used in the reproduction of AD, with the exception of the injection of Aß$_{1-42}$ fragments.

Biomaterial sampling

At the end of the 30-day period of the administration of the analyzed compounds and the referent, the animals were decapitated under chloral hydrate anesthesia; the brain was removed and placed in an ice bath (the temperature no higher than 4°C). Next, the cerebellum was separated, the hemispheres were separated along the central sulcus, and the hippocampus was isolated, then homogenized in a buffer solution consisting of a 0.1% bovine serum albumin solution (Sigma-Aldrich, Germany)+215 mmol of mannitol (Sigma-Aldrich, Germany)+1 mmol of sodium ethyleneglycoltetraacetate (Sigma-Aldrich, Germany)+75 mmol sucrose (Sigma-Aldrich, Germany)+20 mmol of 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (Sigma-Aldrich, Germany). The homogenate was centrifuged (here in after referred to as CM-50 centrifuge, ELMI, Latvia) for 2 min at 1 100 g. The resulting supernatant was divided into two parts. The first aliquot of 700 µl was transferred to Eppendorf tubes and layered with 75 µl of 10% percoll (Sigma-Aldrich, Germany). The resulting mixture was centrifuged for 10 min at 18 000 g. The precipitate was resuspended in 1 ml of an isolation medium and re-centrifuged for 5 min at 10 000 g. In the obtained supernatant (a mitochondrial fraction), the intensity of aerobic and anaerobic respiration, the activity of succinate dehydrogenase (SDH), cytochrome c oxidase (CoX), and citrate synthase (CS) were determined. The second aliquot of the supernatant was used to determine adenosine triphosphate (ATP), mitochondrial hydrogen peroxide (MitoH_2O_2), and apoptosis-inducing factor (AIF) concentration [18, 19].

Evaluation of aerobic respiration intensity

The intensity of an aerobic respiration was determined by the change in the oxygen consumption rate (OCR) in the analyzed medium when 4-(trifluoromethoxy) phenylhydrazonomalononitrile at a concentration of 1 µM/l and pyruvate (15 mmol/l) as a substrate were added. The anaerobic metabolic activity was assessed respirometrically when glucose (15 mmol/l) as substrate and oligomycin (1 µg/ml) were added to the medium. The oxygen consumption was recorded on a laboratory respirometer AKPM 1-01L (Alfa-Bassens, Russia) and expressed in ppm/mg of protein, the content of which was determined by the Bradford method. The reagents used for the resprirometric analysis were provided by Sigma-Aldrich (Germany) [20, 21].

Evaluation of succinate dehydrogenase activity

SDH activity was evaluated spectrophotometrically (here in after referred to as SPh-102 spectrophotometer, Aquilon, Russia) using the succinate-dependent reaction of dichlorophenolindophenol reduction when rotenone was added to the analyzed medium at 600 nm. The reaction medium contained: 40 µM dichlorophenolindophenol, 1 mM KCN, 10 µM rotenone,

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and 50 μM coenzyme Q_2. The reaction was initiated by adding 10 mM succinate. The enzyme activity was expressed in U/mg of protein. Reagents used in the analysis are provided by Sigma-Aldrich (Germany) [22].

Evaluation of cytochrome c oxidase activity

CoX activity was determined by changing the optical density of the cytochrome C (II) oxidation reaction medium in the presence of potassium cyanide at 500 nm. The incubation medium contained cytochrome C (II) – 15 μM and sodium dodecyl sulfate 0.03% solution. The enzyme activity was expressed in U/mg of protein. The reagents, used in the analysis, were provided by Sigma-Aldrich (Germany) [23].

Evaluation of citrate synthase activity

CS activity was evaluated by a spectrophotometric method based on the determination of colored products of the degradation reaction of 5,5'-di-thiobis-(2-nitrobenzoic acid) in the presence of acetyl-CoA and oxaloacetate at 412 nm. The reaction medium contained: 0.1 mM 5,5'-ditiobis-(2-nitrobenzoic acid, 50 mM acetyl-CoA, and 50 mM oxaloacetate. The enzyme activity was expressed in U/mg of protein. The reagents, used in the analysis, were provided by Sigma-Aldrich (Germany) [24].

Evaluation of adenosine triphosphate concentration and apoptosis-inducing factor

The ATP and AIF content in the supernatant of the hippocampus was determined by solid-phase enzyme-linked immunosorbent assay using species-specific reagent kits. The analysis kits were provided by Cloud Clone Corp. (USA, lot no. L231018913). The analysis followed the manufacturer's recommendations. The analytical signal was read using an Infinite F50 microplate reader (Tecan, Austria).

Evaluation of mitochondrial hydrogen peroxide concentration

The MitoH_2O_2 content was determined by the fluorescence method (Hitachi MPF-4 spectrofluorimeter, Japan) using a standard Amplex Red kit (Thermo Fisher Scientific, USA). The MitoH_2O_2 concentration was expressed in mmol/mg of protein.

Statistical analysis

The results were processed by methods of variational statistics using the capabilities of the StatPlus 7.0 Software Package (AnalystSoft Inc., USA, License 16887385). The obtained data were checked for the normality of the distribution according to the Shapiro-Wilk test. Parametric ANOVA methods with the Newman-Keulse post-test and nonparametriccstatic analysis methods – the Kruskal-Wallis test – were used to compare the groups. The differences were considered statistically significant at p <0.05.

RESULTS

Effect of analyzed compounds and donepezil on changes in cellular respiration processes in mitochondrial fraction of AD rats hippocampus

In the course of the study, it was shown that in the NC group of rats in comparison with the SO animals, there was a 60.5% (p <0.05) decrease in the intensity of the aerobic respiration (Fig. 1) with a 164.8% (p <0.05). increase in the anaerobic respiration. During the use of donepezil, there was 24.0% (p <0.05) increase in the activity of the aerobic processes in relation to the untreated rats, while the intensity of the anaerobic respiration did not change significantly. At the same time, in the animals treated with AZBAX 4 and AZBAX6 compounds, the activity of aerobic metabolism exceeded that of the NC group of rats by 83.9 (p <0.05) and 35.9% (p <0.05), respectively, which was accompanied by a decrease in the anaerobic reactions by 27.7 (p <0.05) and 20.6% (p <0.05), respectively. It should be noted that when the AZBAX 4 compound was administered to the animals, the intensity of the aerobic metabolism was higher and the anaerobic metabolism was lower, respectively, than in the rats treated with donepezil, by 48.3 (p <0.05) and 30.8% (p <0.05), respectively. The administration of AZBAX 6 compound reduced the activity of the anaerobic reactions by 24.0% (p *<0.05)* compared to the animals treated with donepezil.

Effect of analyzed compounds and donepezil on changes in enzyme activity in mitochondrial fraction of AD rats hippocampus

As can be seen from the data presented in Table 1, the animals of the NC group showed a decrease in the activity of SDH, CoX and CS in relation to the SO group of rats by 77.0 (p <0.05), 90.5 (p <0.05) and 60.3% (p <0.05), respectively. The use of donepezil contributed to an 80.0% (p <0.05) increase in the activity of CS in comparison with the NC group of the animals, while the activity of SDH and CoX did not change significantly. Against the background of the administration of the AZBAX 4 compound, there was an increase (in relation to the NC group of rats) in the activity of SDH – by 154.3% (p <0.05), CoX – by 180.4% (p <0.05), CS – by 97.8% (p <0.05), whereas when using AZBAX6, the activity of SDH enzymes, CoX and CS increased relative to the NC group indicators by 115.9 (p <0.05), 180.4 (p <0.05), and 71.1% (p <0.05), respectively.

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Effect of the analyzed compounds and donepezil on changes in the ATP concentration in the hippocampal tissue of AD rats

Analyzing the change in the concentration of ATP (Fig. 2) in the hippocampal tissue of rats with AD, it was found that in the NC group of animals, the ATP content decreased by 61.1% (p <0.05) in relation to the SO rats. Against the background of administration of donepezil, AZBAX 4 and AZBAX6 compounds, the level of ATP increased by 68.5% (p <0.05), 112.8% (p <0.05) and 117.1% (p <0.05), respectively, in comparison with the NC the animal group.

When assessing the changes in the concentration of the AIF and rats hippocampus (Fig.3) it was found that in the NC group of animals, this indicator was 3.8 times higher than in the SO rats (p <0.05). At the same time, when donepezil, AZBAX4 and AZBAX6 compounds were administered, the AIF concentration decreased by 24.2% (p <0.05), 46.1% (p <0.05) and 42.8% (p <0.05), respectively, in comparison with the NC group of animals.

Effect of the analyzed compounds and donepezil on changes in the MitoH_2O_2 concentration in the hippocampal tissue of AD rats

In the course of the study, it was shown that in the NC group of rats, the concentration of MitoH_2O_2 exceeded the same indicator of animal SO by 5.4 times (p <0.05). When using AZBAX4 and AZBAX6 compounds, the content MitoH_2O_2 decreased in relation to untreated animals by 63.7% (p <0.05) and 54.8% (p <0.05), while the administration of donepezil did not significantly affect the change in the concentration MitoH_2O_2 in rat hippocampal tissue (Fig. 4).

DISCUSSION

AD is one of the most common neurodegenerative diseases and the leading cause of dementia. In recent years, the incidence of AD does not only decrease, but also tends to increase, which is probably due to an enhance in the life expectancy of the population. Currently, there are no effective kinds of treatments for AD, and available therapeutic strategies are usually focused on symptoms correcting. In many respects, the lack of treatment is due to the not fully established etiopathogenesis of the disease. To explain the specific features of the etiology and pathogenesis of AD, several hypotheses have been put forward, which, as Swerdlow RH points out, should take into account age-related features, pathophysiology of amyloidogenesis and tau pathology, genetic polymorphism of apolipoprotein E, the changes in the cerebral vascular system, inflammations, and insulin resistance [25]. One of the hypotheses that can explain almost all pathophysiological mechanisms of AD identified so far is the "mitochondrial cascade" theory [26].

It is known that the sequential cleavage of the beta-amyloid precursor protein (AßPP) produces a by-product – Aß; its 42-amino-acid oligomeric conformations initiate neurodegeneration. At the same time, AßPP and Aß can partially enter the mitochondria using the external mitochondrial membrane transporter (TOMM). However, this transport is incomplete, since it is hindered by the acid domain of AßPP, resulting in a violation of the architectonics of mitochondrial membranes, a decrease in the intensity of oxidative phosphorylation reactions and the activity of mitochondrial enzymes, in particular CoX [27]. In addition, Aβ disrupts the calcium buffer capacity of mitochondria, thereby aggravating the deficiency of macroergic compounds [28]. Given the important role of mitochondria in the pathogenesis of AD, it is not surprising, that these organelles have become promising pharmacotherapeutic targets.

In this regard, this study was devoted to the evaluation effect of two trimethoxy-substituted monocarbonyl analogues of curcumin on changes in the mitochondrial function of hippocampal cells in rats with experimental AD. Curcuminoids are a class of compounds, with a broad spectrum of a pharmacological activity, including their effect on intracellular signaling pathways regulating the proliferation [29]. An important feature of monocarbonyl curcuminoids, in contraste to full structural analogues of curcumin, is a better pharmacokinetic profile and a higher lipophilicity, which can ensure the penetration of a substances into the BT in significant therapeutic concentrations, thereby increasing the level of effectiveness [30]. In addition, some monocarbonyl curcuminoids may be promising compounds intended for the pathogenetic AD correction. So, Hussain H et al. demonstrated that monomethoxy-substituted monocarbonyl curcumin exerts an antioxidant effect in *in vitro* and in *in vivo* in mice with AD induced by the scopolamine administration. The authors showed that the administration of this compound leads to an increase in the activity of antioxidant enzymes and a decrease in the concentration of malondialdehyde in the hippocampal tissue of the animals. At the same time, the authors associated the antioxidant activity of the analyzed substance with the presence of a methoxy group [31].

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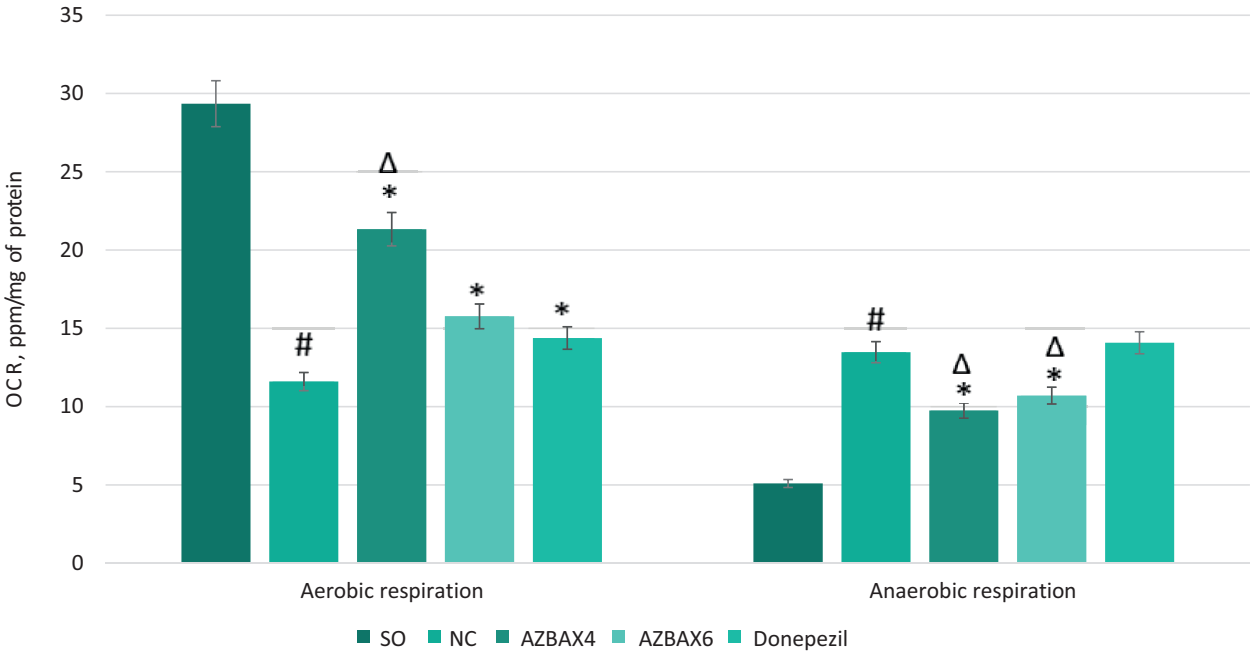

Figure 1 – Influence of analyzed compounds and donepezil on changes in cellular respiration processes in mitochondrial fraction of AD rats hippocampus

Note: SO – sham-operated animals; NC – animals of the negative control group;
– significant relative to SO (Newman–Keulse test, p <0.05); * – significant relative to NC (Newman–Keulse test, p <0.05);
Δ – significant relative to animals treated with donepezil (Newman–Keulse test, p <0.05).

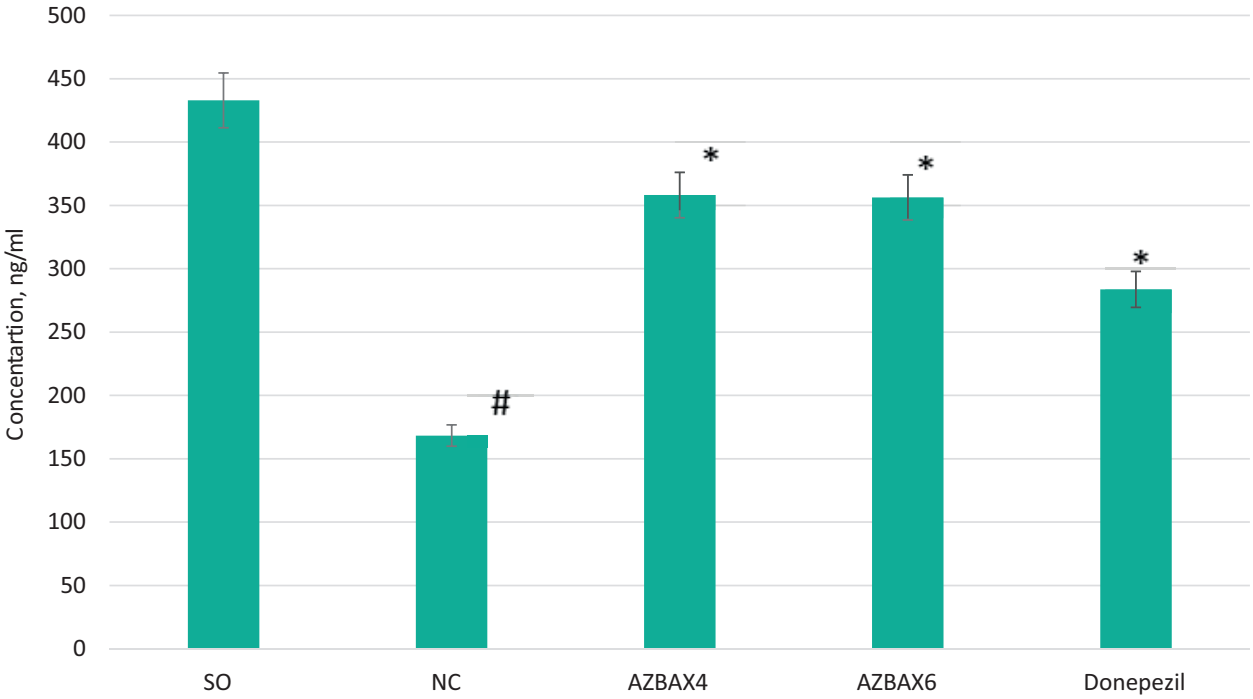

Figure 2 – Effect of analyzed compounds and donepezil on changes in AIF concentration in AD rats hippocampal tissue

Note: SO – sham-operated animals; NC – animals of the negative control group; # – significant relative to SO (Newman–Keulse test, p <0.05);
* – significant relative to NC (Newman–Keulse test, p <0.05);
Δ – significant relative to animals treated with donepezil (Newman–Keulse test, p <0.05).

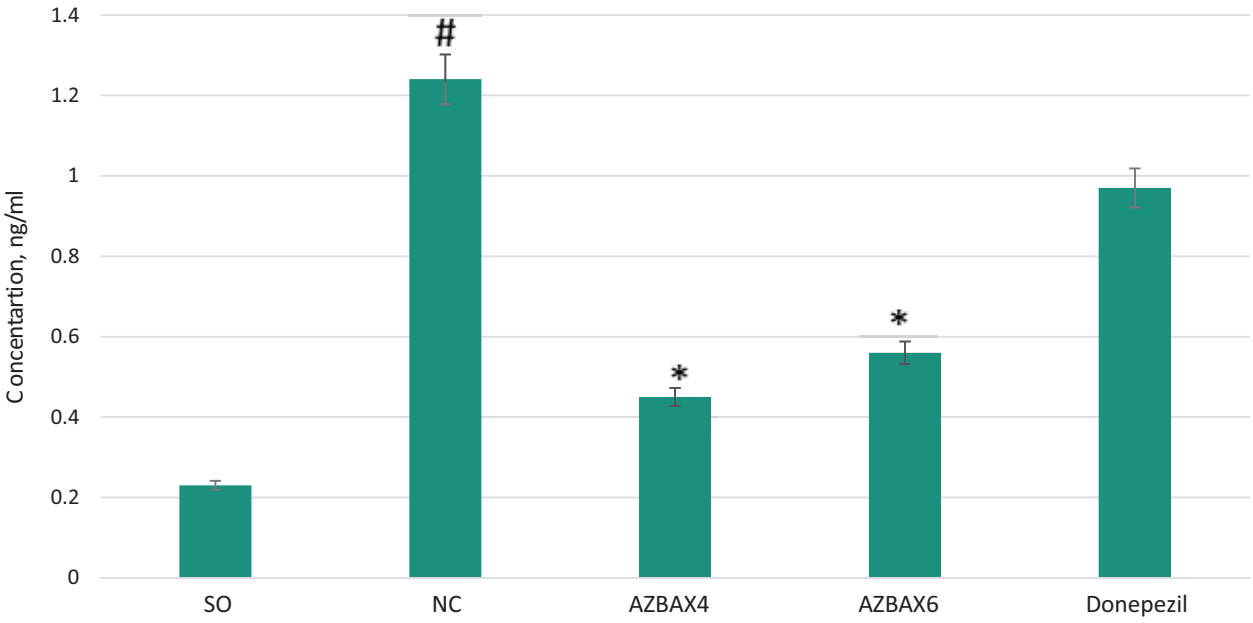

Figure 3 – Effect of the analyzed compounds and donepezil on changes in the AIF concentration i n the hippocampal tissue of AD rats

Note: SO – sham-operated animals; NC – animals of the negative control group; # – significant relative to SO (Newman–Keulse test, p <0.05); * – significant relative to NC (Newman–Keulse test, p <0.05); Δ – significant relative to animals treated with donepezil (Newman–Keulse test, p <0.05).

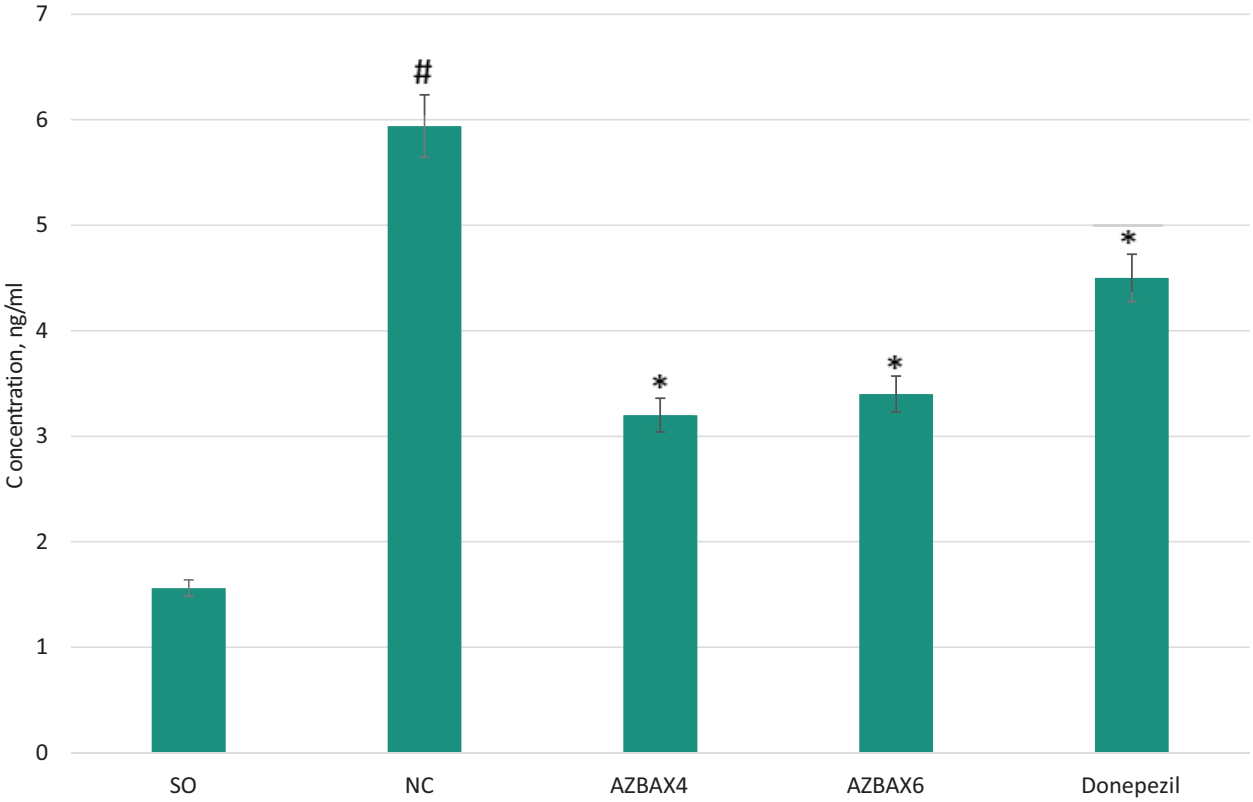

Figure 4 – Effect of analyzed compounds and donepezil on change in the MitoH_2O_2 concentration in AD rats hippocampal tissue

Note: SO – sham-operated animals; NC – animals of the negative control group; # – significant relative to SO (Newman–Keulse test, p <0.05); * – significant relative to NC (Newman–Keulse test, p <0.05); Δ – significant relative to animals treated with donepezil (Newman–Keulse test, p <0.05).

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Table 1 – Effect of analyzed compounds and donepezil on changes in enzyme activity in mitochondrial fraction of AD rats hippocampus

Group	SDH, U/ mg protein	CoX, U/mg protein	CS, U/mg protein
SO	1.19±0.4	4.47±1.12	4.19±0.29
NC	0.27±0.08#	0.43±0.11#	1.66±0.58#
AZBAX4	0.7±0.25*	1.19±0.11*	3.29±0.94*
AZBAX6	0.59±0.31*	1.21±0.15*	2.85±0.97*
Donepezil	0.6±0.16	0.65±0.19	2.99±0.35*

Note: SDH – succinate dehydrogenase; CoX – cytochrome-c-oxidase; CS – citratesynthase; SO – sham-operated animals; NC – animals of the negative control group; # – significant relative to SO (Newman–Keulse test, p <0.05); * – significant relative to NC (Newman–Keulse test, p <0.05).

The study showed that a course administration of trimethoxy-substituted monocarbonyl curcuminoids to the animals contributed to an increase in the intensity of cellular respiration, expressed in an enhance of the aerobic processes intensity and a decrease in the anaerobic ones. A further course of the study allowed us to establish that against the background of the use of the analyzed compounds, there was an increase in the concentration of ATP in the hippocampal tissue of the animals as well as the activity of SDH, CoX and CS, which reflects a significant influence of the studied substances on changes in the mitochondrial function. Thus, an increase in the activity of SDH and CoX may indicate an increase in the processes of mitochondrial biogenesis and autophagy, which lead to the elimination of the damaged mitochondria of neurons that are prone to generating reactive oxygen species and pro-apoptotic molecules, such as cytochrome C or an apoptosis-inducing factor [32]. At the same time, an increase in the CS activity probably indicates an increase in the formation of the intact mitochondria *de novo*, thereby normalizing the aerobic ATP production [33]. It should be noted that when donepezil was administered to the animals, a significant increase in the CS activity, aerobic reactions, and an ATP concentration in relation to the NC group of the animals was observed. At the same time, the use of donepezil did not affect the anaerobic reactions, the activity of SDH and CoX, which is consistent with the results presented by Ye C.Y. e tal. [34]. It is also important that a decrease in the concentration of MitoH_2O_2 and AIF was noted against the background of the use of the studied substances, which reflects a decrease in the intensity of the generation of reactive oxygen species and pro-apoptotic molecules by mitochondria. It is known that MitoH_2O_2 formed as a result of the dissociation of electron transport reactions of the mitochondrial respiratory chain with an electron deletion at the level of complex III, and is one of the most powerful factors provoking an oxidative stress. In this regard, a decrease in its concentration recorded against the background of the administration of the studied substances may indicate not only a decrease in peroxidation processes in the cell, but also a stabilization of the activity of mitochondrial supercomplexes [35].

Taken together, the obtained results indicate that more pronounced changes in the mitochondrial function of hippocampal cells are observed with the administration of the analyzed compounds AZBAX 4 and AZBAX6 than with the use of the reference drug donepezil.

Limitations of the study

This study was performed on female rats in a single-dose mode of the administration of the analyzed compounds, and therefore, in the future, it is necessary to establish possible gender and dose-dependent differences in the effectiveness of the use of the studied substances.

CONCLUSION

The study showed that the course administration of compounds (1E, 4E)-1,5-bis (3,4,5-trimethoxyphenylpenta-1,4-diene-3-one (AZBAX4) and (1E, 4E)-1,5-bis(2,4,6-trimethoxyphenyl) penta-1,4-diene-3 (AZBAX6) to the animals with experimental AD leads to an improvement in the mitochondrial function of hippocampal cells. At the same time, there is an increase in the activity of the mitochondrial enzymes SDH, CS and CoX, the concentration of ATP, as well as some recovery of aerobic metabolism reactions. It is important to note that the effect of the use of the analyzed compounds exceeded that of the administration of the referent-donepezil, which makes them promising for a further study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors made equivalent and equal contributions to the preparation of the publication. All authors confirm that their authorship meets the international ICMJE criteria (all authors made a significant contribution to the development of the concept, conduct of the study and preparation of the article, read and approved of the final version before the publication). Dmitry I. Pozdnyakov – research concept, conducting the experiment, preparing the manuscript; Anastasia A. Vikhor – conducting the experiment, preparing the manuscript; Viktoriya M. Rukovitsina – conducting the experiment, data analysis; Eduard T. Oganesyan – development of the research concept, preparing the manuscript.

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