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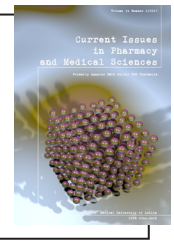

Correction of mitochondrial dysfunction by succinic acid derivatives under experimental cerebral ischemia conditions

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ABSTRACT

The aim of the study. To evaluate the effect of succinic acid derivatives on changes of mitochondrial function in rats under cerebral ischemia conditions.
Materials and methods. In this work, the effect of succinic acid, ethylmethylhydroxypyridine succinate, and acetylaminosuccinic acid at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg (*per os*) on the change of the neuronal mitochondria function was studied. Cerebral ischemia was reproduced by *the Tamura* method. The following parameters were evaluated: changes in aerobic/anaerobic metabolism, mitochondrial membrane potential, the opening rate of the mitochondrial pore of transitional permeability and the activity of apoptotic systems.
Results. During the study, it was found that the use of the test-compounds at doses of 100 mg/kg and 200 mg/kg contributed to an increase in ATP-generating activity, as well as the maximum respiration level and respiratory capacity, while accompanied by a decrease in the intensity of anaerobic metabolism reactions. Also, upon administration of the test succinic acid derivatives, an increase in the mitochondrial membrane potential and latent opening time of the mitochondrial pore transitional permeability were observed. Moreover, the activity of caspase-3 and apoptosis-inducing factor on groups treated by test objects at doses of 100 mg/kg and 200 mg/kg was significantly lower than that in untreated animals.
Conclusion. The studied succinic acid derivatives contribute to the restoration of mitochondrial function in cerebral ischemia conditions, while the most effective dose can be considered to be 100 mg/kg.

INTRODUCTION

Ischemic stroke is a heterogeneous clinical syndrome that is based on the occlusion of cerebral arteries by a thrombus or embolus. Today, the ischemic type of stroke occupies one of the leading places in the structure of mortality and primary disability of the global population [1]. Worldwide, approximately 80-85% of all cerebrovascular pathology is due to ischemic stroke, while about 80% of all people who have this condition are unable to resume work and are subject to social maladaptation [2]. Despite the progress made in understanding the causes, pathogenesis and the principles of rational pharmacotherapy of stroke, the mortality rate from this disease remains quite high and ranges from 47% to 67% [3].

Since the main reason for the development of ischemic stroke is occlusion of the cerebral arteries, the nature of the metabolic and functional disorders that occur when there is a violation of cerebral blood flow and oxygen deficiency largely determine the size of the ischemic cerebral infarction area and the nature of the clinical manifestations of stroke [4]. To date, it has been established that the primary cause of the metabolic shift observed during the manifestation of a stroke is a violation of the functional activity of mitochondria in the ischemic "penumbra" zone [5]. Mitochondria are two-membered cellular organelles that, as a rule, perform three fundamental functions in a cell: energy production,

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regulation of redox and apoptosis reactions [6]. Mitochondria are primarily known as the "energy stations" of cells and contribute to ATP synthesis via the process of electron transport in the mitochondrial respiratory chain and to oxidative phosphorylation (OXPHOS) through the direct use of the reduction equivalents generated in the citric acid cycle [7]. Moreover, the participation of mitochondria in providing antioxidant protection and the regulation of reactions of programmed cell death is important [8].

Research has indicated that a violation of the activity of mitochondria, i.e. mitochondrial dysfunction, is one of the leading elements in the ischemic cascade of brain damage [9]. In addition, it has been noted that mitochondrial damage results in an increase in the formation of reactive oxygen species (ROS) in the cell that is mediated by the dissociation of electron transport reactions at the level of complexes I and II of the mitochondrial respiratory chain and an increase in the activity of NADPH oxidases [10]. Also, electron transport disturbances can be either a cause or a consequence of a decrease in the mitochondrial potential, resulting in a decrease in ATP synthesis and the formation of mitochondrial pore transition permeability (MPTP) [11].

The opening of MPTP is a no return point, after which the initiation of apoptosis processes is irreversible, and activation of both caspase-dependent reactions and caspase-independent pathway is observed, which, with a decreasing in the generation of intracellular macroergs in the ATP form and oxidative modification of the cell structures under the action of ROS leads to an increase in the necrotic area of the brain tissue [12]. Thus, it can be assumed that a targeted effect on neuronal mitochondria aimed at restoring their activity may be a new promising area of cerebroprotective therapy for ischemic stroke.

Derivatives of succinic acid - succinates are currently widely used in medical practice [13]. Succinates have a wide spectrum of neurotropic action, including anxiolytic [14], metabolic [15] and antihypoxic types of action [16], and the neurotropic activity of succinic acid can be mediated by the restoration of the functional activity of mitochondria, as indicated by the study of Nowak G, *et.al*., (2008) [17].

MATERIALS AND METHODS

Experimental animals

The experiment was performed on 110 male Wistar rats weighing 220-230 grams. The animals were obtained from the Rappolovo laboratory animal nursery (Russia, Leningrad Region) and underwent microbiological control and 14-day quarantine. At the time of the study, the rats were kept in macrolon boxes (5 animals each) with free access to water and food. Water and feed were supplied daily; bedding was changed once every three days. Containment conditions were as follows: ambient temperature 22±2°C, relative humidity 60±5%, daily cycle – 12 hours a day, 12 hours a night. Placement, keeping, and all procedures with animals were by Directive 2010/63/EU of the European Parliament and of the council on the protection of animals used for scientific purposes, September 22, 2010. The local ethics committee (protocol No. 1 dated 01/09/2020) approved the research concept and its design.

Test compounds

In this work, we evaluated the effect of some succinic acid derivatives on the change of mitochondrial function under cerebral ischemia conditions, while the test-objects were succinic acid ("Tatkhimpharmpreparaty", RF), ethylmethylhydroxypyridine succinate ("Mexidol", Famrmasoft, RF) and acetylaminosuccinic acid ("Cogitum", Aventis France). The test compounds were administered *per os* 30 minutes after the ischemia reproduction and then for 3 days (once a day) in doses: 50 mg/kg; 100 mg/kg and 200 mg/kg. The choice of doses of the test-compounds was based on previous studies in which some derivatives of succinic acid at a dose of 100 mg/kg had a positive effect on changing the function of mitochondria [18].

Cerebral ischemia rat model

Brain ischemia was modeled by irreversible occlusion of the middle cerebral artery. Operation progress: in anesthetized animals (chloral hydrate 350 mg/kg, intraperitoneally), on the depilated area below and to the right of the eye, the skin was dissected, and muscles were spread. The process of the zygomatic bone was then removed and the skull was exposed. A trepanation hole was made with a drill above the intersection of the middle cerebral artery and olfactory tract, the dura mater was removed and the artery was electrocoagulated, followed by cutting to avoid vascular recanalization and the wound was sutured by layers. The suture was treated by the antiseptic solution – Benzyldimethyl [3-myristoilamine) propyl] ammonium chloride monohydrate); 0.01% solution). The animals were left under a warming lamp until awakening [19]. In this case, the following groups of animals were formed: sham-operated animals (SO) – all sequential operations were applied to this group of animals, except burning out the middle cerebral artery, negative control rats (NC) – group of animals with reproduced ischemia, but without pharmacological correction and groups of rats treated by test compounds. The number of animals in the group was equal to 10 individuals.

Biomaterial sampling

In this study, the animal brain was used as biomaterial. On the 4th day after the operation, in the morning hours (09^{00}-10^{30}), the rats were decapitated under chloral hydrate anesthesia, the cranium was opened and the brain was removed. The brain was then homogenized in a Potter mechanical homogenizer in the media (1 mmol EDTA + 215 mmol mannitol + 75 mmol sucrose + 0.1% BSA solution + 20 mmol HEPES, with a pH of 7.2) at a ratio of brain mass and buffer solution volume of 1 : 7. and were divided into 2 parts. The first part was twice centrifuged in the following modes: 1.400 g → 3 min. at 4°C, after which the re-suspended precipitate was centrifuged at 13000 g → 10 min. The resulting sediment was re-suspended in the isolation medium and removed for respirometric analysis. The second part of the brain homogenate was centrifuged at 10000 g → 5 min. and used for ELISA [20].

Respirometric analysis

Respirometric analysis was carried on the laboratory respirometer AKPM1-01L (Alfa Bassens, Russia). The mitochondrial respiratory function was evaluated by establishing the oxygen consumption in the medium against the injection of mitochondrial respiratory uncouplers. As mitochondrial respiratory uncouplers, this study used: oligomycin 1 μg/ml; 4 – (trifluoromethoxy) phenyl) hydrazono) malononitrile (FCCP-1 μM); rotenone – 1 μM; sodium azide – 20 mmol. The following parameters were defined: ATP-generating ability (difference in oxygen consumption after the addition of FCCP and oligomycin); the maximum level of respiration (according to the difference in oxygen consumption after the addition of FCCP and rotenone) and the respiratory capacity (according to the difference in oxygen consumption after the addition of FCCP and the basal level of oxygen consumption). The activity of glycolysis processes was evaluated when glucose (15 mmol/l) was used as an oxidation substrate. The intensity of glycolysis was determined according to the difference in oxygen consumption after adding glucose and the basal level of oxygen consumption. Glycolytic capacity was assessed according to the difference in oxygen consumption after adding oligomycin and glucose, while glycolytic reserve was established according to the difference in oxygen consumption after adding glucose and sodium azide. During the analysis, the biosample volume was 275 μl, and mitochondrial respiratory uncouplers were injected in a 25 μl volume. Oxygen consumption was determined in ppm with subsequent conversion to the protein concentration in the sample. The protein content was determined using the Bradford method [21,22].

Evaluation of the mitochondrial pore transition permeability (MPTP) opening time

The latent opening time of the MPTP was estimated spectrophotometrically. The incubation medium contained: 0.05 ml of the analyzed resuspended sediment and 20 mM KCl, 0.05 ml of a 0.1 μM solution of cyclosporin A. The resulting mixture was adjusted to 0.2 ml with HEPES buffer solution with a pH of 7.4. The resulting solution was incubated for 25 min at room temperature with constant stirring, after which the optical density in the dynamics was recorded at λ=540 nm. The latent opening time of the mitochondrial pore was evaluated in seconds, recording a decrease in the extinction of the samples from 0.4 to 0.2 [23].

Evaluation of mitochondrial membrane potential

Mitochondrial membrane potential was evaluated by applying the spectrophotometric method. The incubation medium contained: 0.05 ml of the analyzed resuspended sediment and 0.05 ml of 9 μm solution of safranin O. The resulting mixture was adjusted to 0.2 ml with HEPES solution with a pH of 7.4. The optical density of the mixture was recorded at λ=515 nm and λ = 525 nm. The transmembrane electrochemical gradient (ΔΨ) was determined by the difference of sample absorbance:

$$\Delta\Psi = A_{515} - A_{525.} \cdot 1000, \quad [23].$$

ELISA – study

In this work, the concentration of caspase-3 and apoptosis-inducing factor (AIF) was established by enzyme-linked immunosorbent assay in the supernatant of the animal brain. We used species-specific ELISA kits manufactured by *Cloud clone corp.* (USA). The course of the analysis corresponded to the manufacturer's instructions attached to each kit. Results were recorded on an *Infinite F50* microplate reader (Tecan, Austria).

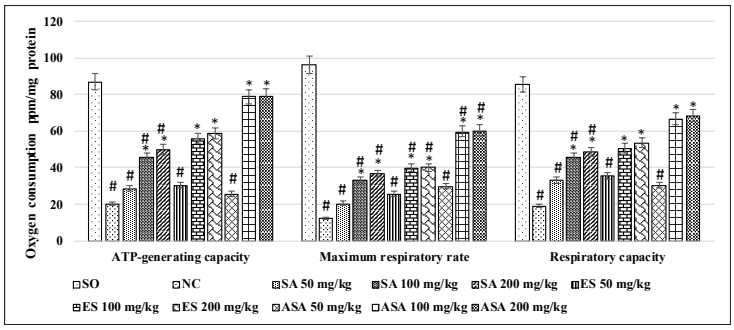
Statistical analysis

Statistical processing of the obtained results was performed in the STATISTICA 6.0 application software package (StatSoft, USA). Data were expressed as M±SEM. A comparison of the mean groups was carried out by the method of one-way analysis of variance with the posterior Newman-Keuls test at a significance level of $p < 0.05$.

RESULTS

The influence of the test compounds on the change in the total respirometric function of mitochondria in conditions of cerebral ischemia

In the course of this block of experimental work, it was found that in NC groups of animals relative to SO rats, a decrease in ATP-generating activity was noted – by 4.3 times ($p < 0.05$); maximum respiratory rate by 7.78 times ($p < 0.05$) and respiratory capacity by 4.5 times ($p < 0.05$). Against the test compounds administration: succinic acid, ethylmethylhydroxypyridine succinate, and acetylaminosuccinic acid at a dose of 50 mg/kg – no significant changes in the overall respirometric function were observed in the animals. However, the use of succinic acid in doses of 100 mg/kg and 200 mg/mg contributed to an increase (relative to the group of rats lacking pharmacological support) of ATP-generating activity by 2.25 times ($p < 0.05$) and 2.47 times ($p < 0.05$); change in the maximum level of respiration of 2.7 ($p < 0.05$) and 2.96 times ($p < 0.05$), respectively, and in respiratory capacity by 2.41 times ($p < 0.05$) and 2.57 times ($p < 0.05$), respectively. The administration of ethylmethylhydroxypyridine succinate and acetyaminosuccinic acid to rats also brought about an increase in ATP-generating activity, as well as the maximal level of respiration and respiratory capacity, while the maximum effect was observed with acetylaminosuccinic acid, the administration of which increased compared with the group of rats lacking pharmacological support, indicators of the overall respirometric function of mitochondria by 3.9 times ($p < 0.05$); 4.86 times ($p < 0.05$) and 3.62 times ($p < 0.05$), respectively. However, no statistically significant differences were found between groups of animals that were treated by acetylaminosuccinic acid at doses of 100 mg/kg and 200 mg/kg, and this regularity was also characteristic by groups of rats treated by ethylmethylhydroxypyridine succinate (Fig. 1).



Note: SO – sham-operated animals; NC – negative control rats; SA – a group of animals treated with succinic acid; ES – group of animals treated by ethylmethylhydroxypyridine succinate; ASA – a group of animals treated by acetylaminosuccinic acid; # – statistically significant relative to the SO group of rats; * – statistically significant relative to the NC group of rats

Figure 1. The effect of the test succinic acid derivatives on the change of the total respirometric function of neuronal mitochondria in rats under cerebral ischemia

The influence of the test compounds on the change in the activity of anaerobic processes in the brain of animals in the cerebral ischemia conditions

When evaluating the activity of anaerobic processes in brain neurons of rats under ischemic conditions, it was noted that in the NC group of animals when compared to the SO rats, an increase in glycolysis intensity – by 7.87 times ($p < 0.05$), was accompanied by a decrease in glycolytic capacity and glycolytic reserve by 7.99 times ($p < 0.05$) and 4.84 times ($p < 0.05$), respectively (Fig. 2). The use of the test derivatives of succinic acid in doses of 100 mg/kg and 200 mg/kg helped to normalize the reactions of anaerobic metabolism, which was manifested in a decrease in the intensity of glycolysis and an increase in glycolytic capacity and glycolytic reserve. Moreover, against the background of succinic acid ethylmethylhydroxypyridine succinate and acetylaminosuccinic acid administration at a dose of 100 mg/kg, the intensity of glycolysis decreased by 43.7% ($p < 0.05$); 37.9% ($p < 0.05$), and 49.8% ($p < 0.05$), respectively, when compared to that of animals lacking pharmacological support. Furthermore, in the use of these compounds at a dose of 200 mg/kg, the intensity of glycolysis in comparison with the NC group of rats was reduced by 42.1% ($p < 0.05$); 37.5% ($p < 0.05$) and 48.1% ($p < 0.05$), respectively (Fig. 2). The value of glycolytic capacity in animals treated by succinic acid, ethylmethylhydroxypyridine succinate, and acetylaminosuccinic acid at a dose of 100 mg/kg increased compared to the same indicator in the rat group NC by 5.5 times ($p < 0.05$); 4.1 times ($p < 0.05$) and 6.2 times ($p < 0.05$), respectively. The glycolytic reserve against the background of the administration of the test compounds at a dose of 100 mg/kg compared with rats lacking pharmacological support also increased: with the use of succinic acid at 2.7 times ($p < 0.05$); ethylmethylhydroxypyridine succinate at 2.6 times ($p < 0.05$) and acetylaminosuccinic acid at 3.2 times ($p < 0.05$). It should be noted that the administration of 50 mg/kg succinate and ethylmethylhydroxypyridine succinate and acetylaminosuccinic acid to the animals did not affect the intensity of anaerobic processes, and there were no statistically significant differences between rat groups treated by the studied compounds at doses of 100 mg/kg and 200 mg/kg not established (Fig. 2).

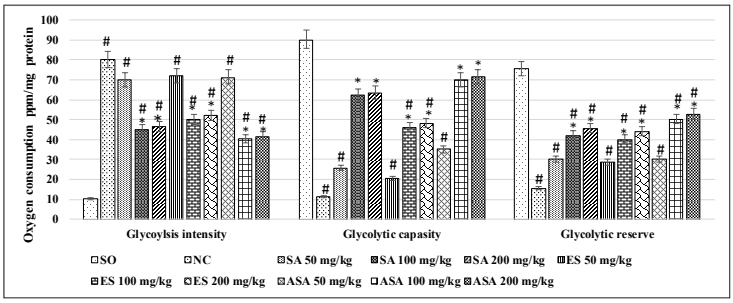


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Figure 2. The effect of the test derivatives of succinic acid on the change in the intensity of anaerobic processes in neurons of rats under cerebral ischemia conditions

The influence of the test compounds on the change in the mitochondrial membrane potential of neuronal mitochondria in animals under cerebral ischemia

In conditions of cerebral ischemia in the NC group of animals, a decrease in the mitochondrial membrane potential (Fig. 3) by 5.3 times ($p < 0.05$) in comparison with the SO group of rats was observed. At the same time, against the background of the succinic acid administration in doses of 100 mg/kg and 200 mg/kg, the mitochondrial membrane potential increased relative to the NC group of animals by 177.4% ($p < 0.05$) and 186.9% ($p < 0.05$), respectively. When using ethylmethylhydroxypyridine succinate, the increase in mitochondrial membrane potential about the group of animals lacking pharmacological support was by 178.6% ($p < 0.05$) – when used at a dose of 200 mg/kg and by 186.9% ($p < 0.05$) – when used in a dose of 200 mg/kg. When rats were treated with acetylaminosuccinic acid at doses of 100 mg/kg and 200 mg/kg, an increase in mitochondrial membrane potential about the NC group of animals by 219.4% ($p < 0.05$) and 226.6% ($p < 0.05$) was observed.

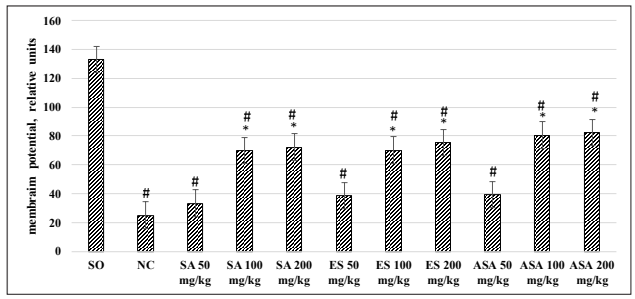

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Figure 3. The effect of the test derivatives of succinic acid on the change of the mitochondrial membrane potential in rats under cerebral ischemia

The effect of the test compounds on the change in the latent time of MPTP opening in animals under cerebral ischemia conditions

When carrying out this block of experimental work, it was found that in rats of the NC group, in comparison

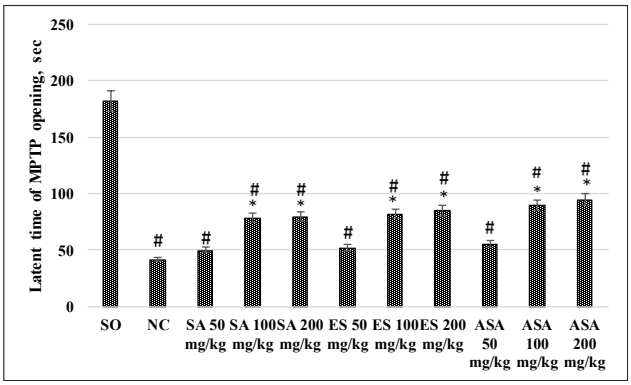

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Figure 4. The effect of the studied derivatives of succinic acid on the change in the latent time of MPTP opening in rats under cerebral ischemia

with the SO group of rats, a decrease in the latent opening time of MPTP (Fig. 4) by 4.3 times ($p < 0.05$) was observed. The administration of succinic acid also contributed to a decrease in the latent time of MPTP formation relative to the NC group of rats at a dose of 100 mg/kg – by 87.4% ($p < 0.05$) and 200 mg/kg – by 90.5% ($p < 0.05$). With the use of ethylmethylhydroxypyridine succinate at doses of 100 mg/kg and 200 mg/kg, a decrease in the time of MPTP opening occurred by 95.7% ($p < 0.05$) and 102.9% ($p < 0.05$) compared with the same indicator in the group of animals deprived pharmacological support. At the same time, the administration of acetylaminosuccinic acid to animals contributed to a decrease in the latent opening time of MPTP compared to the NC group of animals at a dose of 100 mg/kg – by 114.2% ($p < 0.05$) and 200 mg/kg – by 125.4% ($p < 0.05$). It is worth noting that the use of the studied compounds at a dose of 50 mg/kg did not have a significant effect on the latent opening time of MPTP.

The influence of the test compounds on the change in the activity of apoptotic systems in the animals brain under cerebral ischemia conditions

On assessing changes in the activity of apoptotic systems (Fig. 5) in the brain of rats under cerebral ischemia, it was found that the concentration of caspase-3 and AIF in the NC group of animals was by 10.1 times ($p < 0.05$) and 12.1 times ($p < 0.05$) higher than in the rat SO group, respectively. At the same time, against the background of succinic acid administration at doses of 100 mg/kg and 200 mg/kg, a decrease in the content of caspase-3 and AIF in the supernatant of the animal brain by 31.6% ($p < 0.05$) and 35.1%

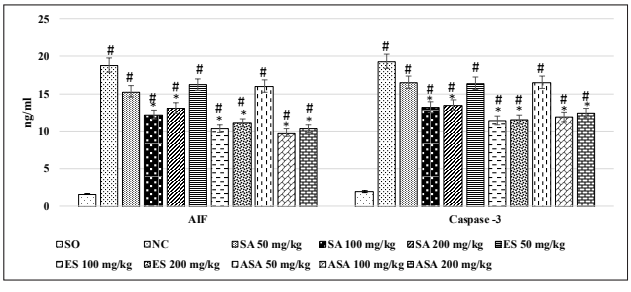

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Figure 5. The effect of the studied succinic acid derivatives on the change of apoptotic systems activity in rat brain supernatant under cerebral ischemia conditions

($p < 0.05$) was observed. Moreover, when using ethylmethylhydroxypyridine succinate at a dose of 100 mg/kg, a decrease in the concentration of caspase-3 by 40.9% ($p < 0.05$) and AIF by 44.7% ($p < 0.05$) was noted, while the administration of this compound at a dose of 200 mg/kg contributed to a decrease in caspase-3 and AIF by 40% ($p < 0.05$) and 41% ($p < 0.05$), respectively. Finally, against the background of administration of acetylaminosuccinic acid at a dose of 100 mg/kg and 200 mg/kg, a decrease in caspase-3 content in rat brain supernatant was observed by 38.3% ($p < 0.05$) and 35.8% ($p < 0.05$) and AIF – by 47.9% ($p < 0.05$) and 44.7% ($p < 0.05$), respectively.

DISCUSSION

Structural and functional disorders of mitochondria play a significant role in the pathogenesis of ischemic-hypoxic damage of organs and tissues, especially those structures that are significantly sensitive to a lack of oxygen and macroergic compounds, for example, brain tissue [24]. It is known that mitochondrial dysfunction is an integral component of the ischemic cascade of cerebral damage and is largely associated with an absence of ATP, which develops during disruption of electron transfer along the respiratory chain of mitochondria and a decrease in proton motive force [25]. Under the prevailing conditions, mitochondria play not so much the role of producers of intracellular energy as they act as consumers of ATP, given the inverse activity of F_1F_0 ATP-synthase aimed at restoring the mitochondrial membrane potential [26]. Moreover, the disconnection of electron transport reactions observed under ischemic conditions is most often identified at the level of complex I – NADH-dehydrogenase, which becomes the main source of ROS in the cell, thereby exacerbating the course of the ischemic process.

In this regard, it can be assumed that biochemical shunting of NADH-dehydrogenase is likely to reduce the degree of mitochondrial damage and, as a consequence, normalize ATP production [27]. In previous studies, it was shown that the use of aspartate and α-ketoglutarate – metabolites of the tricarboxylic acid cycle – under ischemia conditions contributed to the restoration of OXPHOS reactions by increasing substrate phosphorylation. It was noted that most of the studied mitochondria-positive compounds in the organism were metabolized to succinate, which had a shunting effect [28].

This study evaluated the effects of succinic acid, ethylmethylhydroxypyridine succinate, and acetylaminosuccinic acid on changes in mitochondrial function in rat brain neurons under cerebral ischemia. As a result, it was found that the use of the test compounds at doses of 100 mg/kg and 200 mg/kg helped to stabilize the mitochondria respirometric function and reduce the intensity of anaerobic processes. At the same time, increasing the dose from 100 mg/kg to 200 mg/kg did not lead to a significant increase in the pharmacological effect, and at a dose of 50 mg/kg, the studied compounds did not have the proper therapeutic effect, which is probably due to the peculiarities of the absorption of the studied substances and their cell bioavailability [29].

It is also possible that when using excessive doses of the studied substances, there may be an increased generation of reactive oxygen species, resulting in secondary mitochondrial alteration, which limits the use of succinates in high doses, while small doses of these substances do not have the proper therapeutic effect. It is known that succinic acid derivatives are effective exogenous antioxidants and they, like all representatives of this class of substances, have a dose-dependent nature of antioxidant activity, in which when the dose of the compound increases, the antioxidant effect is replaced by a pro-oxidant one [30].

The dose-dependent effect of the studied compounds can also be explained by the regulatory effect of succinates on nitric oxide synthases-enzymes of the NOS system. Inactivation of the inducible isoform of nitric oxide synthase under the action of succinates prevents the generation of peroxynitrite – a strong pro-oxidant with a certain tropicity to mitochondrial membranes. At the same time, the maximum effect of succinic acid derivatives can be obtained when using low doses when administered orally [31].

In this study, it was found that among the studied succinic acid derivatives, the most pronounced effect on changes in mitochondrial function was exerted by the introduction of acetylaminosuccinic acid. This outcome may be explained by the increased bioavailability of this compound in comparison with hydrophilic ethylmethylhydroxypyridine succinate and succinic acid. Moreover, probably acetylaminosuccinic acid penetrates better into the ultrastructure of cells that may increase mitochondriopositive action acetylaminosuccinic acid.

A consequence of the restoration of electron transport reactions may be the normalization of the mitochondrial membrane potential, which was also observed with the use of the studied succinic acid derivatives. In turn, stabilization of the proton motive force prevents the formation of MPTP, which suppresses the initiation of reactions as caspase-dependent and caspase-independent apoptosis pathways [32]. Thus, in the study, it was found that the administration of succinic acid, ethylmethylhydroxypyridine succinate, and acetylaminosuccinic acid to the animals reduced the latent opening time of MPTP, which subsequently contributed to a decrease in the concentration of caspase-3 - the main effector of the caspase-dependent pathway of apoptosis, and an as apoptosis-inducing factor. Furthermore, it is a significant molecule in the initiation and maintenance of caspase-independent reactions [33,34].

It is known that apoptosis is one of the main mechanisms of cell death in the area of ischemic penumbra. At the same time, in contrast to the formed necrotic nucleus, neuronal death in the penumbra region can be observed for several hours (usually up to 72 hours) and proceeds through the mechanisms of caspase-dependent and caspase-independent apoptosis. In this regard, the suppression of apoptotic events under the influence of the studied succinates may favorably affect the functional state of cells in the penumbra region, which in turn prevents an increase in the zone of brain tissue necrosis, thereby realizing a neuroprotective effect [35].

It should be noted that there were no statistically significant differences between groups of animals receiving the test compounds, but the most prognostically positive changes in mitochondrial function were observed with the administration of acetylaminosuccinic acid to the animals.

CONCLUSION

The study showed that the use of succinic acid derivatives under experimental cerebral ischemia dose-dependent restore mitochondrial function. At a dose of 50 mg/kg, there is an insufficient development of the pharmacological effect, while increasing the dose from 100 mg/kg to 200 mg/kg does not significantly increase the therapeutic effect of test-compounds.

CONFLICT OF INTEREST.

The authors declare no conflict of interest.

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