



Experimental study of new derivatives of 3-hydroxypyridine as pharmacological agents for the correction of ischemic brain injury after intracerebral hemorrhage

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Abstract

Introduction: Limiting the action of secondary injury factors can improve the prognosis in acute cerebral accidents. The aim of the investigation is to study the neuroprotective effects of 3-hydroxypyridine derivatives.

Materials and methods: The study was performed in Wistar rats. An intracerebral hemorrhage (ICH) model was used. The animals were once administered intraperitoneally with the test drugs 1 hour before the surgery and on the 1st, 2nd and 3rd days. The registration of behaviors and condition of the animals on days 1, 3, 7 and 14 and the morphological examination of the brain were performed.

Results and discussion: The use of the substances LKhT 4-97 and LKhT 11-02 in the treatment of experimental ICH had a positive effect on the survival rate of the animals and on the resolution rate of pathological signs ($p < 0.05$). Clinical observations were confirmed by the results of analysis of the S100b brain damage marker and morphometry. The efficacy of LKhT 3-15 was largely comparable to that of the reference drug Mexidol. The efficacy of LKhT 01-09 was significantly inferior to that of the reference drug Mexidol. Differences in the neuroprotective effects of the studied substances are related to the metabolism of their various pharmacophores. A hypothetical mechanism for the induction of their neuroprotective effects has been proposed.

Conclusion: Three of the four 3-hydroxypyridine derivatives under study have a neuroprotective effect, which is manifested in a more rapid resolution of pathological symptoms and less pronounced signs of neurodegeneration.

Keywords

hemorrhagic stroke, 3-hydroxypyridines, neuroprotection, the secondary ischemic injury.

Introduction

Hemorrhagic stroke is a spontaneous intraaxial hematoma that is the most severe form of acute cerebrovascular accident (ACVA) (Krylov et al. 2012). More than a third of patients die in the acute period of ischemic brain injury, and at least 84% of survivors remain disabled (Klochikhina et al. 2014). The most common causes of hemorrhagic stroke are hypertonia, arterial and arteriovenous aneurysms, dysemia, and vasculitides (Skidmore and Andrefsky 2002; Wang et al. 2015). As a result of ageing and changes in the racial composition of the world population, as well as a wide use of fibrinolytic therapy, anticoagulants, and oral contraceptives, an increase in hemorrhagic forms in the structure of the acute cerebrovascular events is predicted (Deev et al. 2003; Gusev et al. 2010).

The modern understanding of the morbid physiology of hemorrhagic stroke is based on the concept of the secondary ischemic brain injury (Asdaghi et al. 2011; Qureshi et al. 2016). Bleeding (intracerebral hematoma) leads to the compression of the surrounding brain substance, an increase in intracranial pressure and, as a result, a decrease in cerebral blood flow, impaired cerebral perfusion and activation of ischemic pathobiochemical cascades, such as: changes in the metabolism of glutamate and calcium, free-radical reactions, lipid peroxidation (LPO), excessive generation of nitric oxide, the activation of the astro- and microglial cell pool, immune shifts and local inflammation associated with these changes. The mechanisms of the secondary injury that are activated at the time of hemorrhage involve not only damaged cells in the pathological process, but also intact brain cells, increasing the lesion size (Makhkamov et al. 2015). The size of ischemic brain injury may exceed several times the size of a hematoma, and can be the cause of serious illness of and poor outcomes for a patient (Broderick et al. 1993; Qureshi et al. 2010).

Studies have shown that oxidative stress plays a key part in brain damage after ICH, which leads to irreversible damage to the brain tissue, followed by the disruption of the functioning of the blood-brain barrier and the development of cerebral edema with massive brain cell death (Aronowski et al. 2011).

This link in the pathogenesis of hemorrhagic stroke is targeted by the mechanism of action of 3-hydroxypyridine derivatives. The presence of a 3-hydroxypyridine pharmacophore in the structure of these substances provides their similar antiradical and membranotropic effects. The main presumed mechanisms of the antioxidant action of these substances are oxidation or chelation of catalytically active ions of ferrous iron and the inhibition of free radical LPO reactions, as well as interaction with peroxy (ROO-) and alkoxy radicals (RO-) formed through LPO due to highly mobile hydrogen atom of the phenolic group within molecules (Voronina 2001; Blinov et al. 2011). The difference in acid residues in the composition of the salts is responsible for the differences in the pharmacological properties of

these drugs. These circumstances make it possible to recommend the study of 3-hydroxypyridine derivatives as potential pharmacological agents for the correction of oxidative stress and the prevention of secondary ischemic injury that develop in hemorrhagic stroke.

The aim of the investigation is to study the therapeutic benefits of 3-hydroxypyridine derivatives with potential neuroprotective properties in a rat model of intracerebral hemorrhage.

Materials and methods

Animals

Throughout the study and animal handling process, research ethical principles were applied in accordance with the European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes, CETS No. 123. The experiment was conducted on 250 male Wistar rats. The animals were obtained from a nursery at the Research Institute of Pharmacology of Living Systems, Russia. The animals weighing 250 ± 25 g, with an average index of resistance to hypoxia (selected by means of the model of normobaric hypoxia (Voronina et al. 2012), without any signs of disease, were selected for the study and underwent a 14-day quarantine before the experiment. The rats were kept under the 12-hour daylight conditions with free access to water and food. All the manipulations on the rats were performed under general anesthesia induced with intraperitoneal administration of chloral hydrate. The experimental studies were approved by the Bioethical Committee of Belgorod State National Research University (Minutes №1/19 of 16.01.2019).

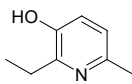
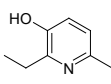
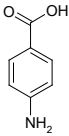
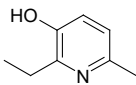
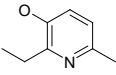
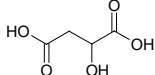
Substances and reference drug

Four new derivatives synthesized at the Department of Chemistry and Technology of Synthetic Drugs and Analytical Control of the All-Union Research Center for Safety of Biologically Active Substances (Kupavna, Russia) were studied. The chemical names of the compounds are given in Table 1.

All the substances are white microcrystalline powders with a slight odor. In accordance with the low solubility of some of the substances under study (LKHT 4-97), in order to objectify the experimental data, all the substances were powdered before the administration and used in the form of a finely-divided suspension prepared on *tween-60*. The resulting suspensions were diluted to the required extent in physiological sodium chloride solution immediately before the start of the experiment. The animals of the control groups received an equal volume of a mixture of physiological solution with *tween-60*.

Mexidol was chosen as a reference drug (*ethylmethylhydroxypyridine succinate*, Pharmasoft, Russia). *Mexidol* is recommended by the Ministry of Health of the Russian Federation and the Federal Medical-Biological Agency

Table 1. New 3-hydroxypyridine derivatives

Laboratory code	Chemical name	Molar mass	Structural formula
LKhT 01-09	3-hydroxy-2-ethyl-6-methylpyridinium N-acetylaminohexanoate	310.63	 $\text{CHCOHN}(\text{CH}_2)_5\text{COOH}$ $\text{CH}_3\text{COHN}(\text{CH}_2)_5\text{COOH}$
LKhT 3-15	3-hydroxy-2-ethyl-6-methylpyridinium 4-aminobenzoate	274.32	 $\text{C}_{15}\text{O}_3\text{N}_2\text{H}_{17}$ 
LKhT 4-97	3-hydroxy-2-ethyl-6-methylpyridinium N-acetylminoacetate	310.63	 $\text{CH}_3\text{COHNCH}_2\text{COOH}$ $\text{CH}_3\text{COHNCH}_2\text{COOH}$
LKhT 11-02	3-hydroxy-2-ethyl-6-methylpyridinium hydroxybutanedioate	207.32	 $\text{C}_{12}\text{O}_6\text{N}_2\text{H}_{17}$ 

as a reference drug for biomedical (preclinical) studies of the antihypoxic activity of drugs (Karkishchenko 2017). This is a derivative of 3-hydroxypyridine, which, like the studied substances, contains the pyridine heterocycle in its structure and is widely used today in neurology in the therapy of stroke.

Design of the experiment

The animals were divided into several groups: 1 – sham operated rats (10 animals), which were anesthetized, with scalping and cranial trepanation performed without destruction of the brain tissue; 2 – untreated animals with hemorrhagic stroke (control group, 40 rats); 3 – animals with hemorrhagic stroke, treated with 74 mg/kg of Mexidol (40 rats); and 5–8 – animals with the simulated pathology, treated with 20 mg/kg of LKhT 01-09, or 20 mg/kg of LKhT 3-15, or 23 mg/kg of LKhT 4-97, or 19 mg/kg of LKhT 11-02, respectively (40 rats in each group). The study drugs were administered to the animals intraperitoneally.

The choice of doses under the study is based on a preliminary study of their toxicity and analysis of the dose-effect relationship (antihypoxic activity) in a model of acute hypoxia with hypercapnia in muscles, followed by the recalculation of doses for rats (according to the interspecies dose scaling).

To scale the equivalent doses of Mexidol for the rat in the study, an interspecies dose scaling was applied (Freireich et al. 1966). For scaling, there was chosen a dose of 800 mg/a day, which is used in the treatment of severe clinical forms of acute cardiovascular events (ACVE) in humans (Vertkin 2016).

The maximum effect of drugs of the 3-hydroxypyridine group the 3-hydroxypyridine group develops 0.58 hours after the moment of administration, and their half-life is 4 hours (Voronina 2000). Thus, the substances under study are administered prophylactically one hour before

the start of the active experiment and on the 1st, 2nd and 3rd days (once a day, at the same time in the morning) (the period of development of ischemic complications).

ICH was simulated by injecting autologous blood into the area of the inner capsule of the right hemisphere of the rat brain (Kleim et al. 2007; Nesterova et al. 2019). This model of experimental hemorrhagic stroke was chosen as the most convenient, technically simple and reproducible.

An analysis of the dynamics of the neurological status of the experimental animals included a quantitative assessment of pathological symptoms using the McGraw Stroke Index, which was modified by I.V. Gannushkina (Gannushkina 1996), the measurement of the grip strength of the limbs using a dynamometer and the assessment of orientation-exploratory behavior to study the motor activity of laboratory animals. The rats were tested before the development of the pathology, as well as on the 1st, 3rd, 7th and 14th days after the ICH simulation (Kolesnichenko et al. 2020). To prevent distortion of the obtained results of the experimental study when analyzing the data of the dynamic assessment of neurological deficit, the indicators of the animals selected for histological examination were censored and were not included in the calculations.

For an objective assessment of the obtained results and their correct interpretation, as well as for a quantitative assessment of a degree of brain damage, the level of S100B protein in the plasma of the experimental animals was measured on the 1st, 3rd, and 7th days of the experiment (Suvaidi et al. 2000).

To identify the assessment of the neuroprotective effects of the substances under the study in the development of secondary ischemic complications after ICH, the size of the perifocal zone of ischemic changes and a morphometric analysis of CA1 neurons in the hippocampal zone were measured on the 3rd day after ICH modeling.

The mortality rate of the animals was recorded daily for 14 days of the experiment.

Hemorrhagic stroke model

Acute autohemorrhagic stroke was simulated in the area of the internal capsule of the right hemisphere of the rat brain (Voronina et al. 2012), according to the method by A. N. Makarenko et al. (Makarenko et al. 2002) in a modification developed by the Research Institute of Pharmacology of Living Systems of Belgorod State National Research University (Russia) (Nesterova et al. 2019). The operation was performed under general anesthesia carried out by intraperitoneal administration of Xyla at a dose of 0.1 ml for sedation; after anxiolysis, the rats were intraperitoneally administered with chloral hydrate at a dose of 300 mg/kg as a basic narcosis. After achieving deep anesthesia, blood was sampled using a syringe from the rat tail vein. Then preoperative showering and linear incision of the scalp in the parietal region were performed. The incision was performed in the frontal plane, followed by hemostasis. The length of the incision was 1.5 cm. Subsequently, skeletization and periosteal separation were performed. A dental burr was used to make a burr hole in the right parietal region, with a diameter of the burr hole being 3 mm. Later, using a puncture needle and a device for stereotactic administration modeled by us, a puncture needle was introduced in the area of the internal capsule (coordinates H=4 mm, L=3.0 mm, A=1.5mm from bregma according to G. Paxinos and C. Watson atlas) to a depth of 3 mm. Then the device was fixed, and a mandrin knife was inserted into the needle; brain tissue was destroyed (the mandrin knife was turned three times clockwise and three times counter clockwise). The mandrin knife was removed, and the rat was aseptically injected with autologous blood taken from the tail vein of the animal in the amount of 0.11 ml/100g of weight. The blood was bolus administered. The effectiveness of the administration was determined by the presence of stem seizures. After that, the puncture needle was removed, the wound was drained, and hemostatic control and layer-by-layer suturing of the wound were performed. The sham operated animals underwent scalping and trepanation.

Assessment of the neurological status of animals

In assessing the neurological status according to the McGraw Stroke Index modified by I.V. Gannushkina, there were identified the rats with mild symptoms (0.5–2.5), moderate symptoms (2.5–5.5) and severe manifestations of neurological disorders (5.5 to 10 points).

Measurement of the animal strength in the grasping test was carried out using a hardware-software complex, designed by A. Yu. Aleynikov in the Student Development laboratory of Robotics. The relative value (specific force) calculated as the ratio of the maximum grip force to the rat body weight was determined as a comparison criterion.

The platform for studying the motor activity of the laboratory animals ACTI-TRACK (PANLAB HARVARD APPARATS) was used to assess the orientation-

exploratory behavior. The rat was tested for 5 minutes in the infrared activity monitor.

Determination of the brain damage marker S100b

For analysis, 5 ml of venous blood was sampled from the experimental rats. Serum was obtained by centrifuging the collected whole blood for 10 minutes at 2500 rpm. The determination of the concentration of S100b was carried out by the method of electrochemiluminescence immunoassay EKLIA, with the help of the test system for quantitative determination of in vitro S100 (S100 A1B and S100). The indices of the group of the non-involved rats were used as the reference value of the marker concentration.

Morphometric assessment of cerebral damage

The randomly selected animals were withdrawn from the experiment on the 3rd day after the start for morphological evaluation. The brain was sampled for histological study. The obtained biomaterial was fixed in a 10% solution of buffered neutral formalin (Biovitrum, Russia) for 24–48 hours. For morphological examination, a hemorrhagic section was excised from the cerebral hemispheres, 2 pieces of 0.2–0.3 cm thick and about 1 cm² in area from each specimen. The drugs were subjected to standard alcohol treatment using a Leica TP 1020 automatic tissue processor (Buffalo Grove, IL) and then, the material was embedded in Histomix paraffin medium (Biovitrum, Russia). The histological sections were made on a rotary microtome HM 340E (MicomInternationalGmbH, Germany) with a thickness of 4–5 μm. The finished sections were stained with hematoxylin and eosin using standard protocols and the techniques on Leica EG 1150 H, Leica RM 2245, and Leica autostainer XL devices. The microscopic studies of the microslides was performed using a Nikon Eclipse Ni microscope and Nis-Elements BR 4.60.00 software.

To assess the comparative efficacy of the studied substances, the morphometry of the peripheral zones of ischemic changes and the morphometric analysis of the CA1 cortex neurons in the hippocampal zone were carried out. The severity of damage and a degree of damage to the hippocampus of rats that had undergone ICH was carried out according to the method by A.I. Chubinidze by the following formulas (1 and 2):

$$\text{Severity of nerve cell damage} = \frac{(GN + MN) \times 100\%}{GN + MN + WN + NN} \quad (1)$$

$$\text{The degree of damage to nerve cells} = \frac{(GN + MN + WN) \times 100\%}{GN + MN + WN + NN} \quad (2)$$

where: GN – grossly modified neurons (with irreversible changes); MN – missing neurons (shadow cells); WN – weakly modified neurons (with reversible changes); NN – normal neurons.

Accounting for animal lethality

The total lethality was calculated as the ratio of dead animals by the end of the experiment (control point) to the total number of animals in the group. Comparative evaluation of survival was assessed by calculating the chi-square value, constructing the tables of survival using the Kaplan-Meier estimator and analyzing the Logrank test.

Statistical analysis

Statistical processing of the findings was carried out in accordance with the guidelines for biomedical statistics (Statistica 10.0 software (Stat Soft Inc., USA) and Excel 2010 (MS OfficeXP, USA). For all the data, the descriptive statistics was applied. The analysis of the significance of intergroup differences was carried out using parametric (Student’s t-test) or non-parametric (Mann-Whitney U-test, chi-square method, Logrank test) methods, depending on the type of distribution. The differences were considered significant at $p < 0.05$.

Results

Dynamics of neurological status

According to the results of a dynamic assessment of the neurologic deficit, the usage of Mexidol led to a more favorable course of the pathology (Fig. 1). A day after the simulation of intracerebral hematoma, the rats that had received Mexidol had less pronounced clinical symptoms. In association with the use of Mexidol, there was no aggravation of the neurological status on Days

3–7, which may indicate the efficacy of this drug to treat ischemic complications of ICH.

The use of Mexidol led to a rapid resolution of neurological semiology than in the control group of the experiment. By the 14th day of the experiment, more than 10% of rats treated with Mexidol had no visible neurological disorders (Fig. 2).

The efficacy of the LKhT 01-09 substance in the experiment turned out to be ambiguous. At the early stage (the 1st day), the drug had no desired therapeutic effect, which led to the death of 50% of the samples (≤ 6 points according to the McGraw scale) (Fig. 1). However, during the period of ischemic complications (Days 3–5) under the influence of LKhT 01-09, the conditions of the experimental animals did not worsen. On the contrary, there was resolution of neurological symptoms, the efficacy of the studied drug being comparable to that of the reference drug Mexidol (Fig. 1). By the end of the experiment (the 14th day), the rats treated with LKhT 01-09 had a more favorable clinical outcome compared to the rats of the control group. However, these changes were inferior to those in the group which had been receiving the reference drug.

The use of the LKhT 3-15 substance allowed the rats to more easily tolerate the severity of the modeled pathology. From the first day of the experiment, the animals of this group showed a smaller neurologic deficit, compared with the control group (Fig. 1). The therapeutic effects of the LKhT 3-15 substance prevented the aggravation of symptoms typical of hemorrhagic stroke on Days 3–5. The use of LKhT 3-15 in the treatment of ICH made it possible to achieve a resolution of the pathological symptoms (on the 7th day, 43% of the group had only mild neurological disorders) and a more favorable outcome by the 14th day when compared to the control group. The clinical effect

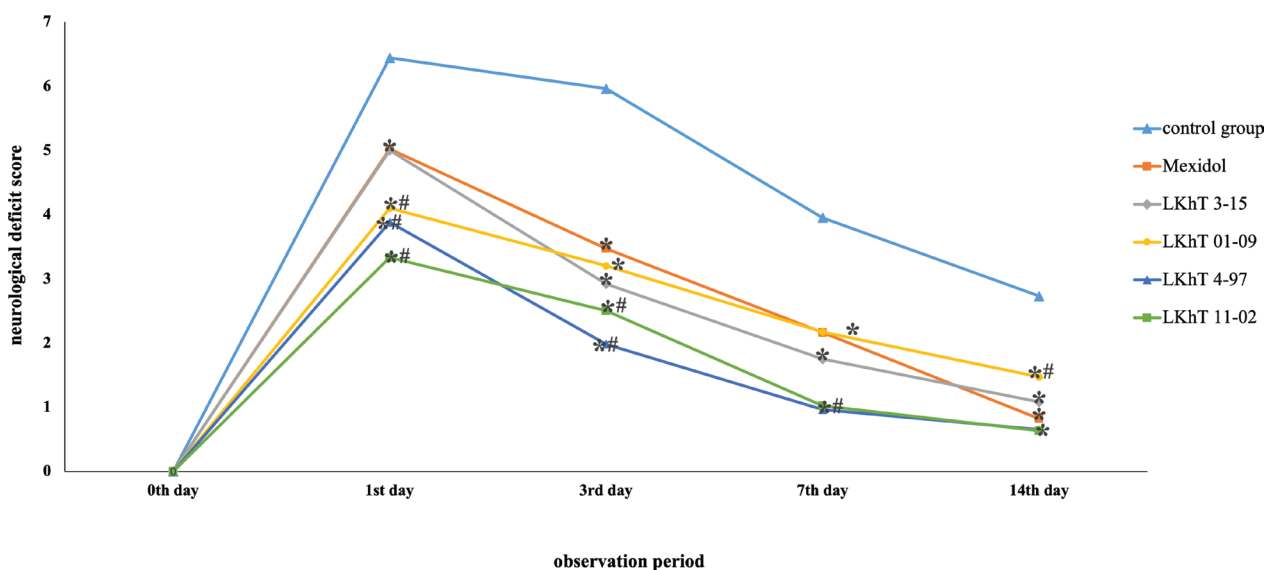


Figure 1. Dynamics of neurological status in rats, according to the McGraw scale modified by I.V. Gannushkina after suffering hemorrhagic stroke while using 3-OP derivatives and without pharmacological correction (n=30). **Note:** * – $p < 0.05$ the differences are statistically significant in comparison with the control group of animals; # – $p < 0.05$ the differences are statistically significant in comparison with the Mexidol reference drug group.

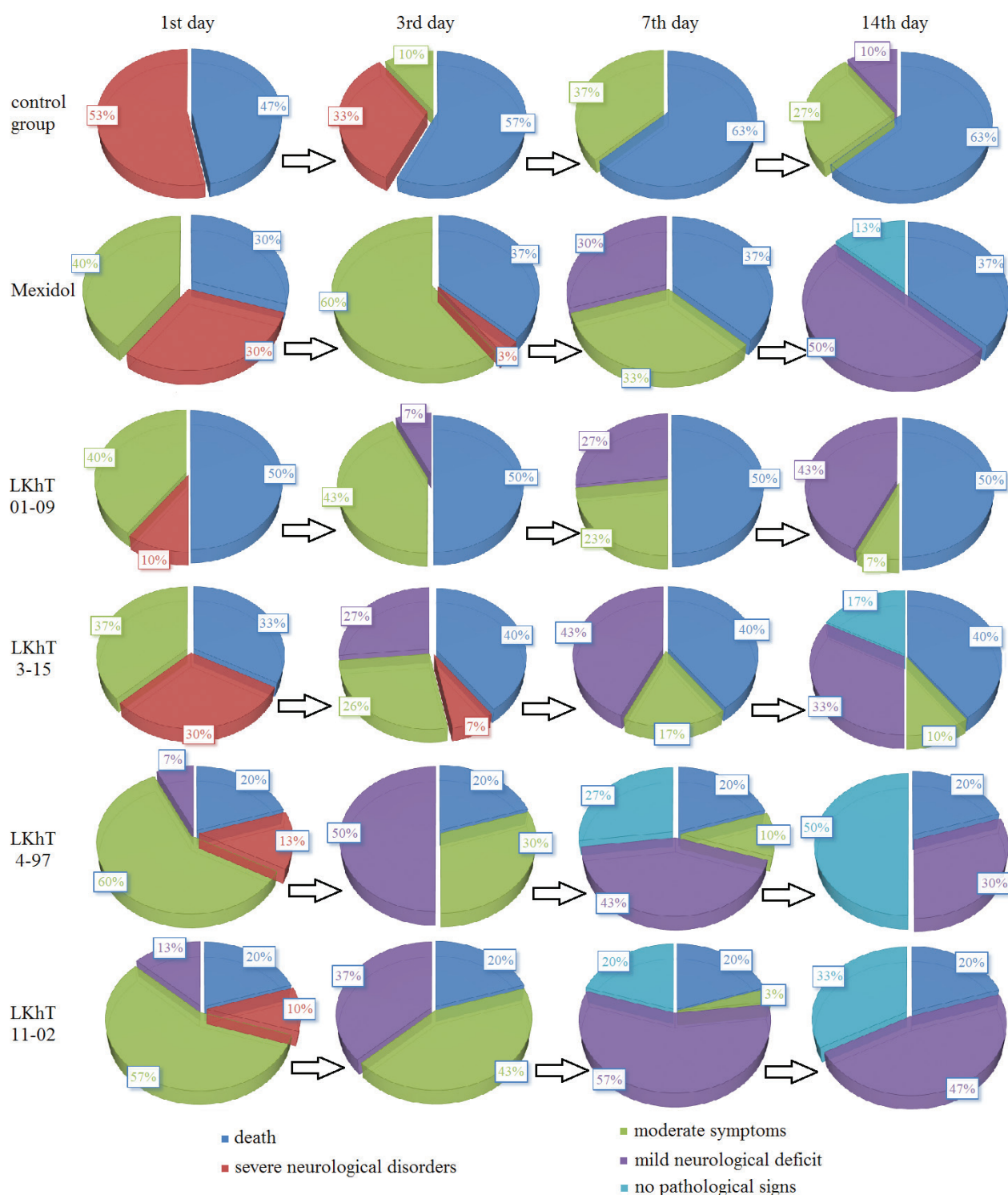


Figure 2. Dynamics of the severity of neurological deficit in rats after hemorrhagic stroke with the use of 3-OP derivatives and without pharmacological correction (n = 30).

of LKht 3-15 was largely comparable to that of Mexidol reference drug. The substance under the study is inferior to Mexidol only in its therapeutic potency for individuals with extremely severe symptoms (≥ 7 points according to the McGraw scale on the 1st day). In this case, its properties were not enough for a more rapid resolution of clinical symptoms.

The most pronounced neuroprotective properties were shown by the substances LKht 4-97 and LKht 11-02. The use of the substance LKht 4-97 and the substance LKht 11-02 in rats with ICH from the 1st day resulted in a more favorable clinical course of the pathology. The rats receiving these substances could not be distinguished from the intact animals on the 7th day of the experiment.

Table 2. Influence of 3-OP Derivatives on Indicators of Muscle Strength of Rats' Paws and General Activity of Animals after hemorrhagic stroke Simulation (M±m)

Indicator under study	Drug used					
	Control group	Mexidol	LKhT 01-09	LKhT 3-15	LKhT 4-97	LKhT 11-02
	Before simulating pathology					
	(n=30)	(n=30)	(n=30)	(n=30)	(n=30)	(n=30)
specific force	6.7±0.5	6.4±0.6	6.6±0.4	6.3±0.5	6.5±0.3	6.2±0.4
total activity	1275±113	1200±148	1253±103	1187±124	1215±93	1301±99
	1st day					
	(n=16)	(n=21)	(n=15)	(n=20)	(n=24)	(n=24)
specific force	2.8±0.3	3.3±0.2	3.1±0.3	2.9±0.4	3.2±0.3	3.1±0.4
total activity	580±84	627±111	599±92	623±146	658±87*	701±99*
	3rd day					
	(n=13)	(n=19)	(n=15)	(n=18)	(n=24)	(n=24)
specific force	3.1±0.2	4.2±0.1*	4.1±0.3*	4.2±0.1*	4.5±0.6*	4.1±0.9*
total activity	556±102	714±84*	700±87*	699±103*	841±84*#	695±134*
	7th day					
	(n=11)	(n=19)	(n=15)	(n=20)	(n=24)	(n=24)
specific force	4.2±0.2	4.7±0.1*	4.6±0.2*	4.5±0.2*	5.5±0.4*#	5.4±0.3*#
total activity	718±82	889±78*	826±57*	901±113*	1097±141*#	1153±132*#
	14th day					
	(n=11)	(n=19)	(n=15)	(n=20)	(n=24)	(n=24)
specific force	4.9±0.3	5.6±0.2*	5.4±0.2*	5.4±0.3*	5.7±0.3*	5.8±0.5*
total activity	856±72	1022±147*	901±46	968±123*	1178±88*	1196±128*

Note: * – p<0.05 the differences are statistically significant in comparison with the control group of animals; # – p<0.05 the differences are statistically significant in comparison with the Mexidol reference drug group.

The persisting mild, neurotic disorders had no significant impact on the life activity and general condition of rats, unlike in those in the Mexidol group, where the similar effect was achieved only by the 14th day (Fig. 1).

Dynamics of changes in muscle strength and general activity

The results of evaluating muscle strength and general activity of the experimental animals during the experiment are presented in Table 2.

Using the substances under the study led to a more rapid recovery of muscle strength in the limbs and activation of orientation-exploratory behavior in comparison with those in the rats that had not received any pharmacological preparations. The substances LKhT 4-97 and LKhT 11-02 were distinguished by a more rapid onset of neuroprotective effects in comparison with Mexidol. By the 3rd day of the experiment, the rats receiving LKhT 4-97 were more active than the rats under the influence of Mexidol. By the 7th day, the LKhT 4-97 and LKhT 11-02 therapy made it possible to achieve a significant restoration of the muscle strength of the limbs and an increase in the general activity of the experimental animals, comparable to the indicators in the Mexidol group by the 14th day.

Dynamics of the level of protein S-100B

The use of 3-OP derivatives in the ischemic brain injury therapy prevented an increase in the S100B concentration in the blood serum of the experimental animals on the

3rd day (Fig. 3). This is evidence of the neuroprotective effects of the studied drugs against the development of secondary ischemic complications of hemorrhagic stroke, and, as a result, it leads to the limitation of the zone of cerebral injury. A significant decrease in the concentration of S100B by the 7th day is evidence of a more rapid activation of the regeneration processes of brain tissue against the background of the use of these drugs.

Kaplan-Meier estimate

The use of Mexidol, as well as LKhT 4-97 and LKhT 11-02, as a pharmacological correction of the experimental hemorrhagic stroke had a positive impact on the survival rate of animals with this pathology (Fig. 4).

Thus, the Mexidol therapy reduces the risk of death in rats with ICH by 1.7 times during the first two weeks, and the usage of both LKhT 4-97 and LKhT 11-02 reduces the risk of death in rats with ICH by more than 3 times.

Morphometric evaluation of the perifocal zone of ischemic changes

When using Mexidol, LKhT 3-15, LKhT 4-97 and LKhT 11-02, the size of the zone of perifocal ischemic damage was significantly smaller than that in the control group (Fig. 5). The best results were recorded when using LKhT 4-97 and LKhT 11-02. Under the impact on these substances, it was possible to achieve a reduction in the zone of pathological changes by more than 38% relative to the control group, and by more than 16% relative to the comparison group.

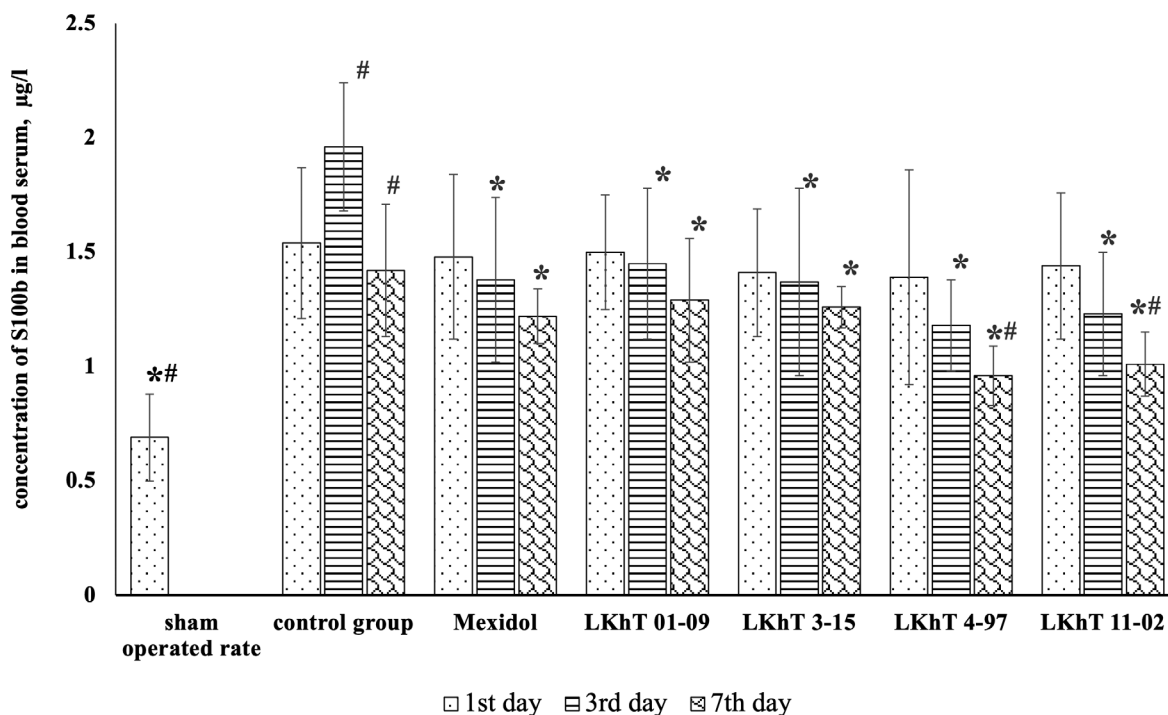


Figure 3. Concentration of the brain damage marker S100B in the blood serum of rats on Days 1, 3, and 7 after suffering hemorrhagic stroke with the use of 3-OP derivatives and without pharmacological correction (n = 10). **Note:** * – p<0.05 the differences are statistically significant in comparison with the control group of animals; # – p<0.05 the differences are statistically significant in comparison with the Mexidol reference drug group.

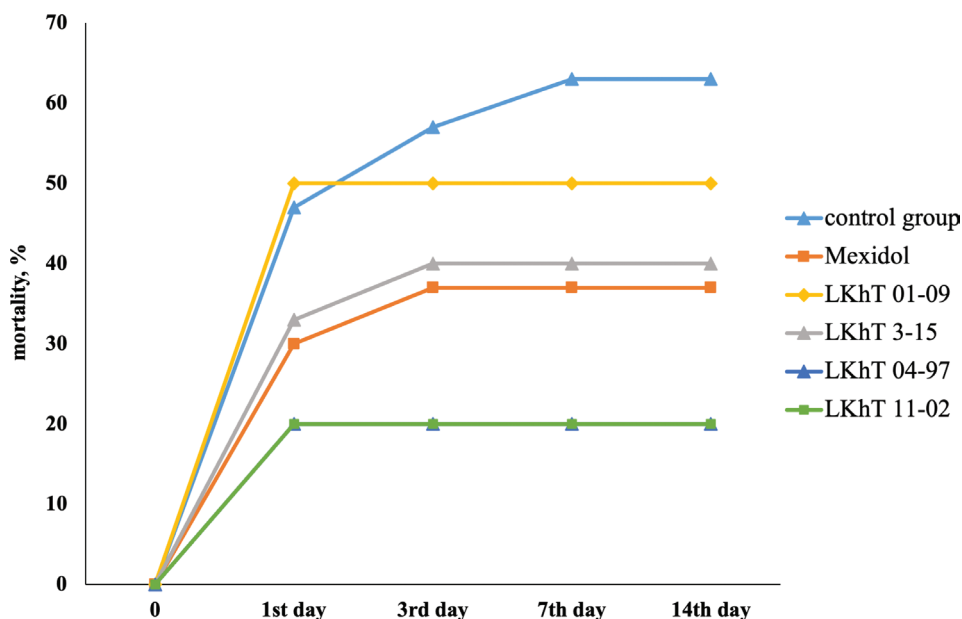


Figure 4. Dynamics of lethal outcomes during the experiment in the control group and against the background of the use of 3-OP derivatives (n=30).

Morphometric study of neurons in the CA1 cortex of the hippocampus zone

When examining the hippocampus of the experimental rats, the most severe ischemic changes (to the point of necrobiosis) were observed in the control group (Table 3).

When using of LKht 3-15, LKht 4-97, LKht 11-02 and Mexidol, the affect to the hippocampal neurons was less evident than that in the control. While using LKht 01-09, there was a significant degree of damage to the CA1 zone of the hippocampus ($24.45 \pm 6.12\%$ of modified neurons) and a high severity of damage to nerve cells of neurons, with prevailing necrobiotic changes ($55.81 \pm 4.32\%$).

Table 3. Severity of CA1 Lesion in the Hippocampus Region (M±m, n=30)

Group	Severity of neuron defects, %	Degree of damage of neurons, %
Control	28.32±5.16	64.21±6.25
Mexidol	19.64±3.31*	45.0±3.77*
LKhT 01-09	24.45±6.12	55.81±4.32
LKhT 3-15	16.22±4.87*	53.26±5.07
LKhT 4-97	13.87±8.11*	37.24±6.67*
LKhT 11-02	15.21±4.36*	39.43±4.81*

Note: * – $p < 0.05$ the differences are statistically significant in comparison with the control group of animals.

Discussion

According to the experimental data obtained, LKhT 4-97 and LKhT 11-02 have the most pronounced neuroprotective effects when simulating ICH in rats. The use of these substances in ICH therapy had a positive effect on both the survival rate of animals and the resolution rate of pathological signs. The clinical observations are confirmed by the results of the S100B brain injury marker and morphometry.

The most pronounced antihypoxic and neuroprotective effects of LKhT 4-97 (3-hydroxy-2-ethyl-6-methylpyridinium N-acetylaminoacetate), in comparison with those of Mexidol, are probably associated with the presence of an acidic residue of N-acetylaminoacetic acid in the structure of this substance (Fig. 6). As a donor of a biologically important amino acid – glycine (aminoacetic acid – a product of hydrolysis of N-acetylaminoacetate), LKhT 4-97 interrupts the pathobiochemical processes that develop in the brain tissue in response to damage in hemorrhagic stroke. According to the literature data, the compensation for the deficient aminoacetic acid reduces oxidative stress: inhibits the synthesis of ROS (Froh et al.

2002), contributes to the maintenance of high levels of glutathione (Sekhar et al. 2011), superoxide dismutase (Yuldashov et al. 2013) and catalase (Abduvaliev et al. 2013), which are the key components of cell antioxidant defense. Glycine in the acute phase of ischemic complications, by activating specific receptors, has a cytoprotective action, preventing apoptosis and necrosis (McCarty et al. 2009). Glycine promotes vasodilation in the brain (Podoprigrora et al. 2005); in ischemia, it reduces LPO and the formation of malondialdehyde (Meyer et al. 2006), reducing the secondary injury of vessels. The presence of the pharmacophore of N-acetylaminoacetic acid in the structure of LKhT 4-97 determines the high antioxidant, anti-ischemic and cytoprotective activities of this 3-OP derivative when treating cerebral injuries developing in hemorrhagic stroke.

LKhT 11-02 (2-ethyl-6-methyl-3-hydroxypyridine hydroxybutanedioate) is malate. The most pronounced pharmacological effects of LKhT 11-02 11, when compared to those of the reference drug Mexidol, are probably due to the higher bioavailability and efficacy of malates (Fig. 6), when compared to succinates in cerebrovascular pathologies. Malate easily passes through the blood-brain barrier. The uniqueness of the mechanism of action of malate has been proven: during hypoxia, it undergoes the metabolism of ATP formation 2.5 times more often than succinate (Blinov et al. 2008).

The efficacy of the use of LKhT 3-15 (3-hydroxy-2-ethyl-6-methylpyridinium 4-aminobenzoate) when treating experimental hemorrhagic stroke was in many respects comparable to that of Mexidol. The use of the LKhT 3-15 substance allowed the rats to tolerate the severity of the simulated pathology more easily and achieve a rapid resolution of pathological symptoms. The studied substance was inferior to Mexidol only in therapeutic efficacy for individuals with extremely severe

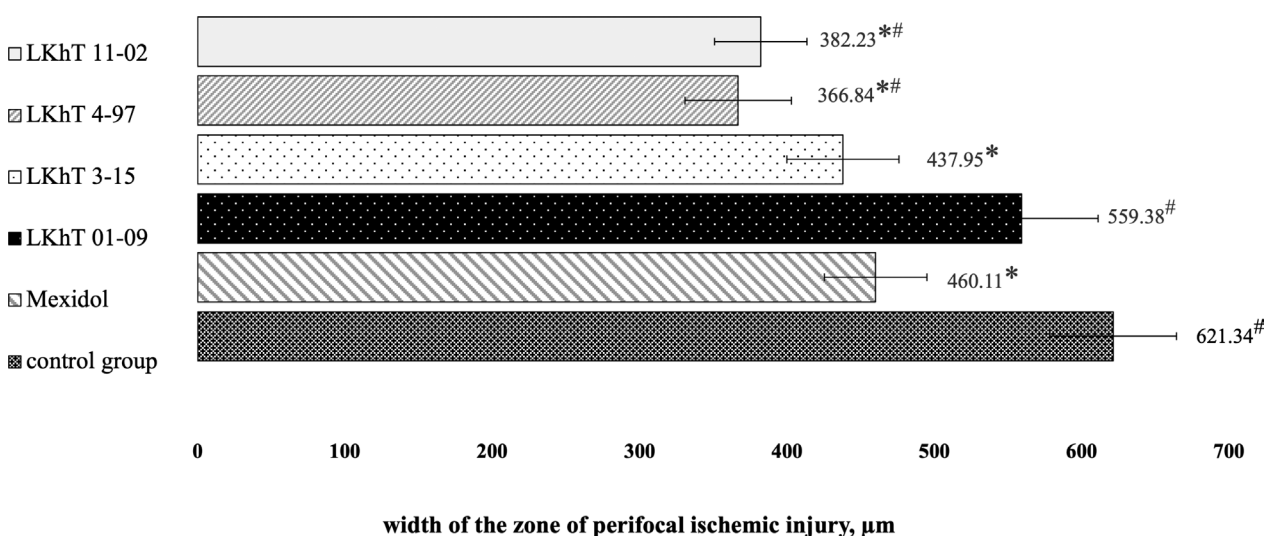


Figure 5. Width of the zone of perifocal ischemic brain damage in rats with simulated intracerebral hemorrhage with the use of 3-OP derivatives and without pharmacological correction (n=30). Note: * – $p < 0.05$ the differences are statistically significant in comparison with the control group of animals; # – $p < 0.05$ the differences are statistically significant in comparison with the Mexidol reference drug group.

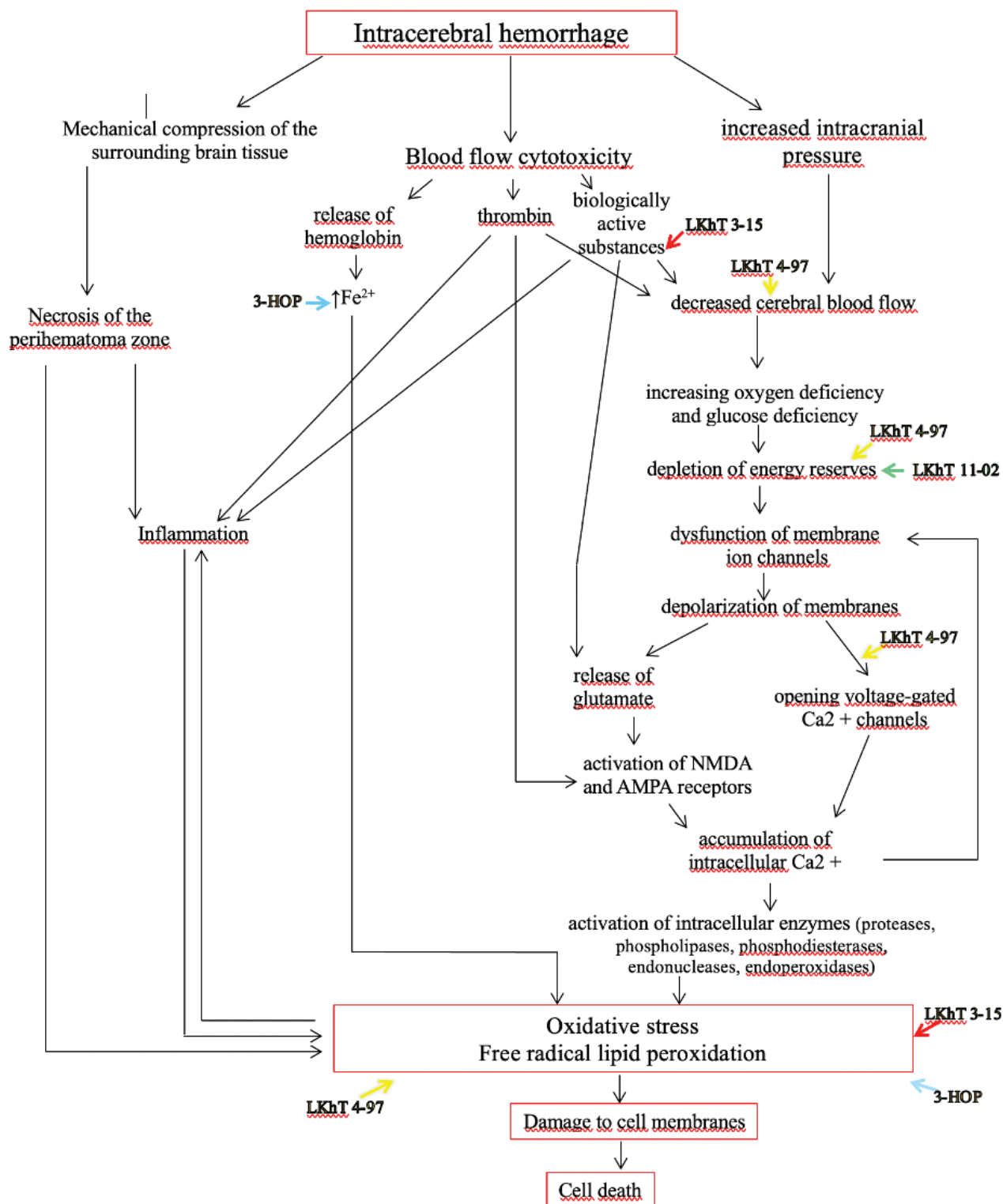


Figure 6. The fragments of a hypothetical mechanism for the realization of the neuroprotective effects of ethylmethylhydroxypyridine of 4-aminobenzoate (LKht 3-15), ethylmethylhydroxypyridine of N-acetylaminoacetate (LKht 4-97), and ethylmethylhydroxypyridine of hydroxybutanedioate (LKht 11-02) on a hemorrhagic stroke model. **Note:** blue arrow – site of application of the action of the 3-hydroxypyridine pharmacophore, yellow arrow – site of application of the N-acetylaminoacetic acid pharmacophore (LKht 4-97), green arrow – site of application of the malic acid pharmacophore (LKht 11-02), red arrow – site of application of the pharmacophore of *para*-aminobenzoic acid (LKht 3-15).

symptoms (≥ 7 points according to the McGraw scale on the 1st day). In this case, its properties turned out to be insufficient for an effective resolution of the clinical symptoms of a previous hemorrhagic stroke. Besides,

the use of LKht 3-15 had no significant influence on the survival rate of rats with this pathology.

The pharmacological effects of LKht 3-15 are probably based on the activity of the hydrolyzates of this substance,

namely, 3-hydroxypyridine and para-aminobenzoic acid (Fig. 6). The para-aminobenzoic pharmacophore probably had a positive effect on cellular metabolism, inhibiting the action of histamine and serotonin, and exerting an antioxidant effect (Drozd et al. 2000; Akberova et al. 2001). However, the antioxidant activity of para-aminobenzoic acid is not sufficient to prevent the accumulation of the LPO-malondialdehyde in the brain tissues. The pharmacofoms of 3-hydroxypyridine and para-aminobenzoic acid showed no significant synergism in the development of antioxidant and neuroprotective effects in the ICH model in rats. In this connection, the efficacy of this substance when correcting secondary brain injury in the model of hemorrhagic stroke in rats was lower than that of Mexidol.

The least effective treatment for experimental hemorrhagic stroke was the use of LKhT 01-09 (3-hydroxy-2-ethyl-6-methylpyridinium N-acetylaminohexanoate). At an early stage (the 1st day of the experiment), the drug produced no desired therapeutic effect, which led to the death of 50% of the sample and the survival of individuals with only moderate clinical symptoms (≤ 6 points according to the McGraw scale). However, during the period of ischemic complications (Days 3–5) under the influence of LKhT 01-09, the condition of the experimental animals did not worsen, but on the contrary, some regression of neurological deficit was observed. This was confirmed by the analysis of the biochemical marker of cerebral damage S100B (stabilization of its concentration was recorded on the 3rd day). By the end

of the experiment (the 14th day), the rats receiving LKhT 01-09 had a more favorable clinical outcome compared to the rats of the control group. However, these changes were significantly inferior to those in the group receiving Mexidol. The low neuroprotective activity of LKhT 01-09 in the model of hemorrhagic stroke in rats is probably due to the formation of aminohexanoic acid as a result of the metabolism of the N-acetylaminohexanoate pharmacophore. An antifibrinolytic agent, used to rapidly limit intracerebral hematoma and prevent rebleeding, likely aggravated microcirculatory disorders in brain tissue, preventing putative antioxidant (Saliev et al. 2020) and neuroprotective effects from unfolding to the full (Arlt 2010).

Conclusion

Analyzing the ICH model in rats, it was found that three of the four new derivatives of 3-hydroxypyridine: LKhT 3-15 (20 mg / kg), LKhT 4-97 (23 mg / kg) and LKhT 11-02 (19 mg / kg) – have neuroprotective properties. The most effective substances – LKhT 4-97 and LKhT 11-02 – outperform the drug Mexidol by intensity of action.

Conflict of interests

The authors of this paper report no conflicts of interest.

References

- Abduvaliev AA, Zokhidov ZhM, Nishantaev MK, Karimova ShF, Alimkhodzhaeva NT (2013) Effect of glycine on catalase activity in the dynamics of experimental myocardial infarction. The Successes of Modern Natural Science [Uspekhi Sovremennogo Estestvoznaniya] 9: e11. [in Russian]
- Akberova SI, Stroeva OG, Magomedov NM, Babaev NF, Galbinur AP (2001) Comparative assessment of the antioxidant activity of para-aminobenzoic acid and emoxypin in the cornea and lens (experimental studies). Annals of Ophthalmology [Vestnik Oftalmologii] 4: 25–29. [PubMed] [in Russian]
- Arlt AV (2010) Effect of aminocaproic acid on cerebral blood flow. Pharmacy [Farmatsiya] 1: 45–47 [in Russian]
- Aronowski J, Zhao X (2011) Molecular pathophysiology of cerebral hemorrhage: secondary brain injury. Stroke 42(6): 1781–1786. <https://doi.org/10.1161/STROKEAHA.110.596718> [PubMed] [PCM]
- Asdaghi N, Hameed B, Saini M, Jeerakathil T, Emery D, Butcher K (2011) Acute perfusion and diffusion abnormalities predict early new MRI lesions 1 week after minor stroke and transient ischemic attack. Stroke 42(8): 2191–2195. <https://doi.org/10.1161/STROKEAHA.110.611376> [PubMed]
- Blinov DS, Sernov LN, Balashov VP, Blinova EV, Pivkina LV, Gogina ED, Van'kova LV, Vertyankin MV, Boiko GG, Krasilina TV (2008) Antiarrhythmic activity of the new domestic antihypoxant Etoxidol. Bulletin of Arrhythmology 53: 41–43. <https://doi.org/10.1007/s10517-012-1581-3> [PubMed] [in Russian]
- Blinov DS, Sernov LN, Balashov VP, Blinova EV, Pivkina LV, Gogina ED, Van'kova LV, Vertyankin MV, Boiko GG, Krasilina TV (2012) Antiischemic activity of new domestic antioxidant 3-hydroxypyridine etoxidol derivative. Bulletin of Experimental Biology and Medicine 152(5): 583–586. <https://doi.org/10.1007/s10517-012-1581-3> [PubMed] [in Russian]
- Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G (1993) Volume of intracerebral hemorrhage. A powerful and easy-to-use predictor of 30-day mortality. Stroke 24: 987–993. <https://doi.org/10.1161/01.str.24.7.987> [PubMed]
- Deev AS, Zakharushkina IV, Konovalov OV (2003) Etiological factors, course and outcomes of ischemic strokes in young people. Neuroimmunology [Neyroimmunologiya] 1(2): 44–45. [in Russian]
- Drozd NN, Makarov VA, Miftakhova NT, Kalugin SA, Stroeva OG, Akberova SI (2000) Antithrombotic activity of para-aminobenzoic acid. Experimental Clinical Pharmacology 3: 40–44. [PubMed] [in Russian]
- Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemotherapy Reports 50(4): 219–244. [PubMed]
- Froh M, Thurman RG, Wheeler MD (2002) Molecular evidence for a glycine-gated chloride channel in macrophages and leukocytes. American Journal of Physiology. Gastrointestinal and Liver Physiology 283 (4): 856–863. <https://doi.org/10.1152/ajpgi.00503.2001> [PubMed]

- Gannushkina IV (1996) Rat neurological deficit assessment scale. *Neuropathology and Psychiatry [Nevropatologiya i Psikhiatriya]* 1: e14. [in Russian]
- Gusev EI, Konovalov AN, Skvortsova VI (2010) *Neurology: National Guidelines*. Geotar-media, Moscow, 1040 pp. [in Russian]
- Karkishchenko NN, Karkishchenko VN, Shustov EB, Kapanadze GD, Reviakin AO, Semenov HH, Bolotova VT, Dulya MS (2017) Biomedical (preclinical) study of antihypoxic activity of drugs. Methodical recommendation. *FMBA of Russia MR*, 21–44. [in Russian]
- Kleim JA, Boychuk JA, Adkins DL (2007) Rat models of upper extremity impairment in stroke. *ILAR Journal* 48 (4): 374–384. <https://doi.org/10.1093/ilar.48.4.374> [PubMed]
- Klochikhina OA, Stakhovskaya LV (2014) Analysis of the epidemiological indicators of stroke according to the data of the territorial-population registers of 2009–2012. *Korsakov Journal of Neurology and Psychiatry [Zhurnal Nevrologii i Psikhiatrii imeni S.S. Korsakova]* 114 (6): 63–69. [PubMed] [in Russian]
- Kolesnichenko PD, Scheblykina OV, Nesterova NI, Scheblykin DV, Nesterov AV, Pokrovsky MV, Zhuchenko MA, Tverskoy AV, Reznikov KM (2020) Additive neuroprotective effect of 3-hydroxypyridine and human erythropoietin derivatives in a rat model of hemorrhagic stroke. *Pharmacy and Pharmacology* 8(3): 169–180. <https://doi.org/10.19163/2307-9266-2020-8-3-169-180>
- Krylov VV, Dashyan VG, Burov AS, Petrikov SS (2012) *Hemorrhagic Stroke Surgery*. Medicine, Moscow, 336 pp. [in Russian]
- Makarenko AN, Kositsin NS, Pasikova NV, Svinov VV (2002) Simulation of local cerebral hemorrhage in different brain structures of experimental animals. *Journal of Higher Nervous Activity* 52(6): 765–768. [PubMed] [in Russian]
- Makhkamov KE, Kuzibaev ZhM (2015) Prediction and diagnosis of the progression of secondary brain injury in patients with traumatic intracerebral hemorrhage. *Emergency Medicine Bulletin [Byulleten' Ekstrennoi Meditsiny]* 1: 36–39. [in Russian]
- McCarty MF, Barroso-Aranda J, Contreras F (2009) The hyperpolarizing impact of glycine on endothelial cells may be anti-atherogenic. *Medical Hypotheses* 73(2): 263–264. <https://doi.org/10.1016/j.mehy.2008.12.021> [PubMed]
- Meyer KF, Martins JL, Freitas Filho LGd, Oliva ML, Patrício FR, Macedo M, Wang L (2006) Glycine reduces tissue lipid peroxidation in hypoxia-reoxygenation-induced necrotizing enterocolitis in rats. *Acta Cirúrgica Brasileira* 21(3): 161–167. <https://doi.org/10.1590/s0102-86502006000300008> [PubMed]
- Nesterova NI, Shchablykina OV, Kolesnichenko PD, Nesterov AV, Shchablykin DV, Popova IA, Yakovlev DV (2019) Neuroprotective effects of taurine and 3-hydroxypyridine derivatives in the intracerebral hemorrhage model in rats. *Research Results in Pharmacology* 5(3): 87–94. <https://doi.org/10.3897/rpharmacology.5.36988>
- Podoprigora GI, Nartsissov YR, Aleksandrov PN (2005) Effect of glycine on microcirculation in pial vessels of rat brain. *Bulletin of Experimental Biology and Medicine* 139(6): 675–677. <https://doi.org/10.1007/s10517-005-0375-2> [PubMed]
- Qureshi AI, Palesch YY, Martin R, Novitzke J, Cruz-Flores S, Ehtisham A, Ezzeddine MA, Goldstein JN, Hussein HM, Suri MF, Tariq N (2010) Effect of systolic blood pressure reduction on hematoma expansion, perihematomal edema, and 3-month outcome among patients with intracerebral hemorrhage: results from the antihypertensive treatment of acute cerebral hemorrhage study. *Archives of Neurology* 67(5): 570–576. <https://doi.org/10.1001/archneurol.2010.61> [PubMed] [PCM]
- Qureshi AI, Palesch YY, Barsan WG, Hanley DF, Hsu CY, Martin RL, Moy CS, Silbergleit R, Steiner T, Suarez JI, Toyoda K, Wang Y, Yamamoto H, Yoon BW (2016) Intensive blood-pressure lowering in patients with acute cerebral hemorrhage. *The New England Journal of Medicine* 375(11): 1033–1043. <https://doi.org/10.1056/NEJMoa1603460> [PubMed] [PCM]
- Saliev T, Baiskhanova D, Beznosko D, Begimbetova D, Umbayev B, Nurgozhin T, Fakhraiyev I, Tanabayev B, Pavalkis D (2020) A new insight on the radioprotective potential of epsilon-aminocaproic acid. *Medicina (Kaunas)* 56(12): e663. <https://doi.org/10.3390/medicina56120663> [PubMed] [PMC]
- Sekhar RV, McKay SV, Patel SG, Guthikonda AP, Reddy VT, Balasubramanyam A, Jahoor F (2011) Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. *Diabetes Care* 34(1): 162–167. <https://doi.org/10.2337/dc.10-1006> [PubMed] [PMC]
- Skidmore CT, Andrefsky J (2002) Spontaneous intracerebral hemorrhage: epidemiology, pathophysiology, and medical management. *Neurosurgery Clinics of North America* 13(3) 281–288. [https://doi.org/10.1016/s1042-3680\(02\)00019-0](https://doi.org/10.1016/s1042-3680(02)00019-0) [PubMed]
- Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes Jr DR, Lerman A (2000) Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 101(9): 948–954. <https://doi.org/10.1161/01.cir.101.9.948> [PubMed]
- Vertkin AL (2016) Effective tissue anti-ischemic therapy of vascular diseases of the brain of various origins. *Cardiovascular Therapy and Prevention [Kardiovaskulyarnaya Terapiya i Profilaktika]* 15(2): 69–78. <https://doi.org/10.15829/1728-8800-2016-2-69-78> [in Russian]
- Voronina TA (2001) Antioxidant Mexidol. The main neuropsychotropic effects and mechanism of action. *Psychopharmacology and Biological Narcology [Psikhofarmakologiya i Biologicheskaya Narkologiya]* 1: 2–12. [in Russian]
- Voronina TA (2000) Mexidol, main effects, mechanism of action, application. *Methodical recommendations*. Voronezh, 45 pp. [in Russian]
- Voronina TA, Ostrovskaya RU, Garibova TL (2012) Guidelines for the preclinical study of drugs with a nootropic type of action. In: *Guidelines for Conducting Preclinical Studies of Drugs. Part 1*. Grif K, Moscow, 944 pp. [in Russian]
- Wang X, Arima H, Yang J, Zhang S, Wu G, Woodward M, Muñoz-Venturelli P, Lavados PM, Stapf C, Robinson T, Heeley E, Delcourt C, Lindley RI, Parsons M, Chalmers J, Anderson CS; INTERACT2 Investigators (2015) Mannitol and outcome in intracerebral hemorrhage. *Stroke* 46(10): 2762–2767. <https://doi.org/10.1161/STROKEAHA.115.009357> [PubMed]
- Yuldashov SI, Yuldashev NM, Alimkhodzhaeva NT, Karimova ShF (2013) Superoxide dismutase activity in the dynamics of experimental myocardial infarction and during treatment with glycine. *The Successes of Modern Natural Science [Uspekhi Sovremennogo Estestvoznaniya]* 9: 75–76. [in Russian]

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