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Original Article

Investigation of Taurine Derivative Magnesium-Bis-(2-Aminoethanesulfonic)-Butadioateon Alleviation of Neurological Defects in Simulated Hemorrhagic Stroke in Rats

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Abstract

Stroke or ischemia is caused by a blockage in a specific blood vessel that partially or completely reduces the blood flow to the brain. Nutritional factors such as antioxidants and healthy eating patterns are important variables in preventing stroke. Molecular composition properties such as molecular binding and screening can be used to evaluate the specific activity and morphological changes. The present study aimed to evaluate the effectiveness of pharmacological correction of the consequences of a hemorrhagic stroke in rats with a new derivative of taurine magnesium-bis-(2-aminoethanesulfonic)-butadioate. The animals (n=170) were divided into four groups as follows: 1) control group (n=20), 2) group 2 suffered a hemorrhagic stroke without pharmacological correction (n=50), 3) group 3 (n=50) underwent simulation of hemorrhagic stroke received Taurine at the dose of 50 mg/kg, 4) Group 4 underwent simulation of hemorrhagic stroke with correction of hemorrhagic stroke with magnesium-bis-(2-aminoethanesulfonic)-butadioate at the dose of 150 mg/kg (LKHT 3-17) (n=50). Hemorrhagic stroke was induced by transfusing autologous blood into the parietal lobe of the right hemisphere of the brain. Lethality, neurological status, locomotor, and exploratory behavior, as well as the morphological pattern of the brain damage, were assessed on the 1st, 3rd, and 7th days after the pathology simulation. Neurological deficit was determined in animals by the McGrow stroke index scale. The locomotor and exploratory behavior was evaluated using the Acti-track software and hardware complex. Two criteria were considered when assessing morphological changes in the brain: the average thickness of the cerebral cortex (in micrometers) and the number of neurons without degenerative changes. LKHT 3-17 (150 mg/kg) and taurine (50 mg/kg) reduced lethality by 1.7 and 1.36 times, respectively, on the 3rd day after stroke compared to that of the control ($p<0.05$). In parallel, a neurological deficit was effectively corrected LKHT 3-17 and taurine to 5.3±0.8 and 6.5±0.9, respectively, on the 1st day in contrast to the control of 8.1±0.7 points. The locomotor and exploratory behavior was significantly different on the 7th day and was accompanied by a significant increase in total activity under the influence of LKHT 3-17 to 491 conventional units (CU) compared to the control of 110 conventional units. On the 1st day, the thickness of the cortex was 1943.7±44.08 µm, and 1491.0±38.61 µm in the control and LKHT 3-17 groups, respectively. The number of neurons without neurodegenerative changes prevailed in LKHT 3-17 group (18.7±4.32), and the lowest number was observed in the group without pharmacological correction of the pathology (14.3±3.78). The taurine derivative magnesium-bis-(2-

aminoethanesulfonic)-butadioate, which is a combination of the amino acid, magnesium ion, and succinic acid, decreases the neurological deficits, lethality, and enhances the locomotor and exploratory behavior in experimental hemorrhagic stroke in rats. The effect of the studied medication on the dynamics of molecular pathophysiological mechanisms occurring in the cell requires additional research.
Keywords: Hemorrhagic stroke, Neuroprotection, Taurine

1. Introduction

Hemorrhagic stroke is caused by a brain hemorrhage that occurs with a ruptured cerebral artery which accounts for a high percentage of deaths (1). Hemorrhage due to rupture of cerebral arteries, cerebral aneurysms, and weak-walled rupture can cause hemorrhagic stroke. Hemorrhagic stroke is due to bleeding into the brain by the rupture of a blood vessel. Hemorrhagic stroke may be further subdivided into intracerebral hemorrhage (ICH) and subarachnoid hemorrhage (SAH) (2). The Russian Stroke Association reports that 40000 intracerebral hemorrhages are annually registered in Russia. The relevance of the problem of cerebrovascular pathology is currently important due to the high lethality and severe disability of patients. Developing a new medication for treating this pathology is an emerging issue. Cerebroprotective agents significantly affect neurology, neurosurgery, ophthalmology, and pediatrics. Recently, a large number of studies have been published on various types of neuroprotective medications which reveal the importance of the issue (3-7).

Preclinical studies of new molecular compounds should include molecular docking and screening, assessment of specific activity, and morphological changes (8, 9). Therefore, the amino acid taurine is widely used in ophthalmological practice, but not applicable in the Russian Federation, neurology, and neurosurgery. Taurine is the most abundant amino acid which is involved in acute ischemic stroke through several mechanisms such as bile acid composition, osmotic regulation, anti-inflammatory, anti-oxidation, membrane stabilization, and calcium homeostasis. Taurine can reduce ischemia/hypoxia and glutamate by suppressing endoplasmic reticulum stress and maintaining intracellular homeostasis (10). The pharmacological effect of this substance in the correction of cerebrovascular pathology has been mentioned in foreign sources (11). Taurine has several unique advantages, including endogenous biological function, low molecular weight, which can easily cross the blood-brain barrier into the brain parenchyma, and specific pharmacokinetic properties and taurine metabolism in animals and humans (10). The taurine molecule stimulates a process called remyelination, which is essential for repairing damaged nerves. Taurine is mostly obtained from seafood, livestock, and poultry (12). This amino acid is involved in the composition of bile salts, blood pressure regulation, anti-oxidation, and anti-inflammatory (13-15). Taurine may lower cholesterol and blood pressure. Researchers have reported an inverse ecological association between the groups of urinary taurine excretion, taurine replacement, and body mass index, blood pressure, cholesterol levels, and stroke mortality rates (16-20).

Taurine reduced glutamate-mediated toxicity by reducing oxidative stress and [Ca^{2+}] overloading simulated stroke in rats. It also blocked two of the three UPR paths. Although the mechanisms underlying the action of taurine against endoplasmic reticulum stress (ER stress) and UPR pathways have been being studied yet, it is known that taurine deficiency is associated with ER stress (11). Several pieces of evidence conclude that taurine is effective in the treatment of acute stroke in rats (11, 21). Other properties of taurine, such as anti-oxidant, anti-inflammatory, or osmoregulatory, may also contribute to its neuroprotective mechanism in ischemic stroke (11, 22).

The present study aimed to evaluate pharmacological correction of the consequences of hemorrhagic stroke by new taurine derivative magnesium-bis-(2-aminoethanesulfonic)-butadioate under laboratory code LKHT 3-17 in rats.

2. Material and Methods

2.1. Animals

The studied substance magnesium-bis-(2-aminoethanesulfonic)-butadioate was synthesized in the Russian Scientific Center for the Safety of Bioactive Substances (StarayaKupavna, Russia) and provided for experiments by Sofia Ya. Skachilovaunder laboratory code LKHT 3-17. The experiments were performed on 170 white male Wistar rats weighing 250 ± 25 g without external signs of diseases and were quarantined for 10-14 days. The animals were kept for conducting preclinical studies in the Russian Federation following the rules of laboratory practice (GOST Z 51000.3-96 and 51000.4-96) and the Order of the Ministry of Health of the Russian Federation No.267 of 19.06.2003 "On Approval of the Rules of Good Laboratory Practice" (GLP), in compliance with the international recommendations of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1997).

The animals were kept in a special room with a day/night cycle changing after 12 hours and provided with a standard laboratory diet.

2.2. Research Design

LHT 3-17 (150 mg/kg) and an equimolar dose of taurine (50 mg/kg) were administered intraperitoneally 60 minutes before pathology simulation. Anesthesia was performed by sequential intramuscular administration of Xylazalum 0.1 ml for premedication and 1% Zoletil solution at the dose of 1 mg/100 g 45 minutes before the surgery.

The animals were divided into the following groups:

1. Intact group (n=20)
2. Simulation of hemorrhagic stroke without pharmacological correction (n=50)
3. Correction of hemorrhagic stroke with taurine at a dose of 50 mg/kg (n=50)
4. Correction of hemorrhagic stroke with magnesium-bis-(2-aminoethanesulfonic)-butadioate at a dose of 150 mg/kg (n=50).

2.3. Simulation of the Hemorrhagic Stroke

Hemorrhagic stroke was simulated according to the previously described method in the Patent №2721289 (13).

After proper and deep anesthesia, autologous blood was sampled from the tail vein, in a volume of 0.1 ml/100 g.

A 3 mm diameter burr hole was made in the skulls of the rats using dental boron (NTI (Germany)). Cerebral tissue is decomposed in three clockwise and then counterclockwise rotations by rotary knife. Then, the knife was removed and autologous blood was injected under a pressure of 110 mm Hg.

The lethality, neurological deficit, locomotor activity, and morphological examination were assessed on the 1st, 3rd, and 7th days after simulating the hemorrhagic stroke.

2.4. Assessment of the Neurological Status of Animals

Neurological deficit in the animals was determined by the McGrow stroke index scale. The severity of the condition was assessed by the sum of the corresponding points. The number of rats was as follows: with mild symptoms (up to 2.5 points on the stroke index scale) – sluggishness of movements, weakness of limbs, hemiparesis, tremor, circling behavior; and with severe neurological disorders (from 3 to 10 points) - paresis and paralysis of the limbs, as well as lateral position (23).

2.5. Evaluation of the Locomotor and Exploratory Behavior

Evaluation of the locomotor and exploratory behavior was determined using Acti-track (Panlab Harvard Apparatus) software and hardware. The rat was placed in an infrared frame for 5 minutes. The data was automatically recorded without the participation of an operator.

2.6. Assessment of Brain Changes by Histological Examination

Two pieces of brain with a thickness of 0.2-0.3 cm were examined after 24-hour fixation in 10% neutral formalin for morphological evaluation. The section was made in the frontal plane through the entire brain of each animal. The samples were subjected to standard histological processing using Leica TP 1020 device, after which sections with a thickness of 4-5 microns were prepared and stained with hematoxylin and eosin, using standard protocols and techniques on LeicaEG 1150 H, Leica RM 2245, and Leica autostainer XL devices. Two criteria were statistically evaluated: the average thickness of the cerebral cortex in microns and the number of neurons without degenerative changes.

2.7. Statistical Processing

Descriptive statistics were applied to all the data. Shapiro-Wilk normality test was performed. In the case of normal distribution, the mean value (M) and standard error of the mean (m) were calculated. The outliers at each time point were identified using Grubbs's test. If for any sample, the value of Z was greater than the critical value for the specified measurements N, this sample was excluded from further calculations. The median (Me) and quartile range (QR) were calculated in cases of non-normal distribution. The inter-group differences were analyzed using parametric (Student's t-test) or nonparametric (Mann-Whitney U test) methods, depending on the type of distribution. The differences were determined at the 0.05 significance level. The statistical analysis was performed using the Statistica software (version 10.0).

3. Results

3.1. Lethality

It was found that the mortality rate in the control group was 44% on the 1st day after hemorrhagic stroke simulation (Figure 1). On the 3rd day, the mortality rate increased to 68%, and on the 7th day increased to 78%. The mortality rate in the group administered with LKHT 3-17 (150 mg/kg) was 8%, 24 hours after the pathology simulation. On the 3rd day, the lethality rate increased to 40% and it was 44% on the 7th day. In the comparison group of taurine at the equimolar dose (50 mg/kg), the lethality rate on the 1st day was 14%. On the 3rd day, the lethality rate increased to 50%, and it was 60% by the 7th day.

3.2. Assessment of the Neurological Status of the Animals

According to the data obtained in the experimental groups with simulated hemorrhagic stroke, LKHT 3-17 (150 mg/kg) significantly reduces the severity of neurological disorders in comparison with the control group and the reference drug - taurine (50 mg/kg) (Figure 2).

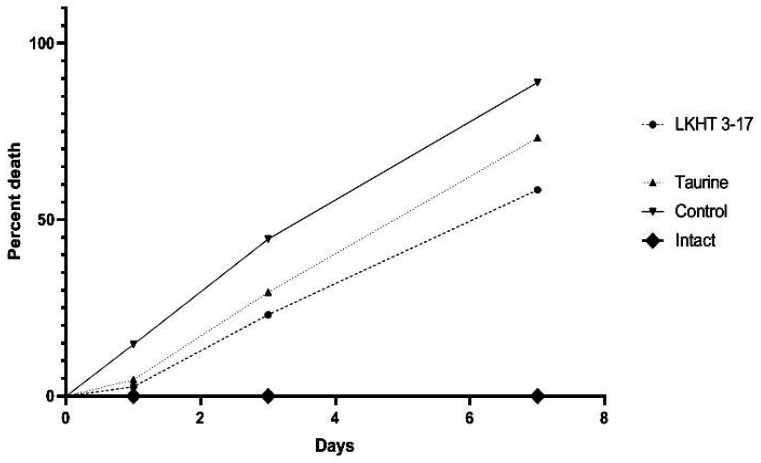

Figure 1. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the lethality dynamic in experimental hemorrhagic stroke.
Note: The effect of the studied medications on the survival of rats in simulated hemorrhagic stroke in the experiment

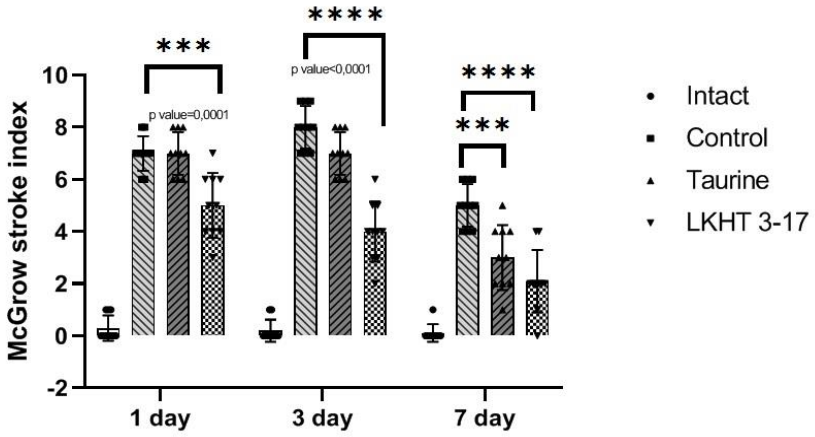

Figure 2. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of neurological status according to McGrow stroke index scale on the 1st, 3rd, and 7th days in experimental hemorrhagic stroke
Note: Dynamics of the severity of neurological injuries in the studied groups on the scale of assessing the severity of neurological deficit in meningitis, meningoencephalitis (by the average score in the group)

3.3. Evaluation of the Locomotor and Exploratory Behavior

The locomotor activity of the animals was assessed in the IR Actimeter test which indicated that those who were administered with the test medication were significantly more active throughout the experiment compared with the group without pharmacological correction of hemorrhagic stroke (Table 1).

3.4. Evaluation of brain changes by histological examination

According to the recorded data in this study, the highest thickness of the brain cortex was observed in animals without pharmacological correction, and the lowest in the group of experimental medication 24 hours after the simulation of the hemorrhagic stroke (Figure 3 and 5).

The highest number of neurons without degenerative changes was observed in LKHT 3-17 group (18.7±4.32) compared to that of the control group (14.3±3.78) (Figure 5, 6, and 7). At further control points, the following dynamic of morphological changes was observed: the thickness of the brain cortex reached its maximum in the control group on the 3rd day (2424.0±49.2) (Figure 4 and 6).

Table 1. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the locomotor and exploratory behavior of the animals with experimental hemorrhagic stroke on the 1st, 3rd, and 7th days (M±m)

Day	Group	Total activity (cu)	Stereotypies (cu)	General locomotor activity (cu)	Distance covered (cu)
1st day	Intact group	1172±226	100±22	1072±210	2287±608
	Control	355±42	29±4.3	326±21	649±37
	Taurine	277±17***	23±3.12	255±17	548±42*
	LKHT 3-17	570±27***	46±5.7***	524±49***	1008±56***
3rd day	Control	332±16	29±2.86	303±32	661±57
	Taurine	526±26	41±3.15	484±42	990±87
	LKHT 3-17	555±32***	42±2.94*	513±16*	1078±55***
7th day	Control	110±13	9±0.11	101±14	216±11
	Taurine	313±41*	27±1.03	286±15	559±45
	LKHT 3-17	491±32***	36±2.65***	454±21*	963±79***

Note: *** p<0.0001 * p<0.0015 - the differences are statistically significant compared to the control group of the corresponding time point

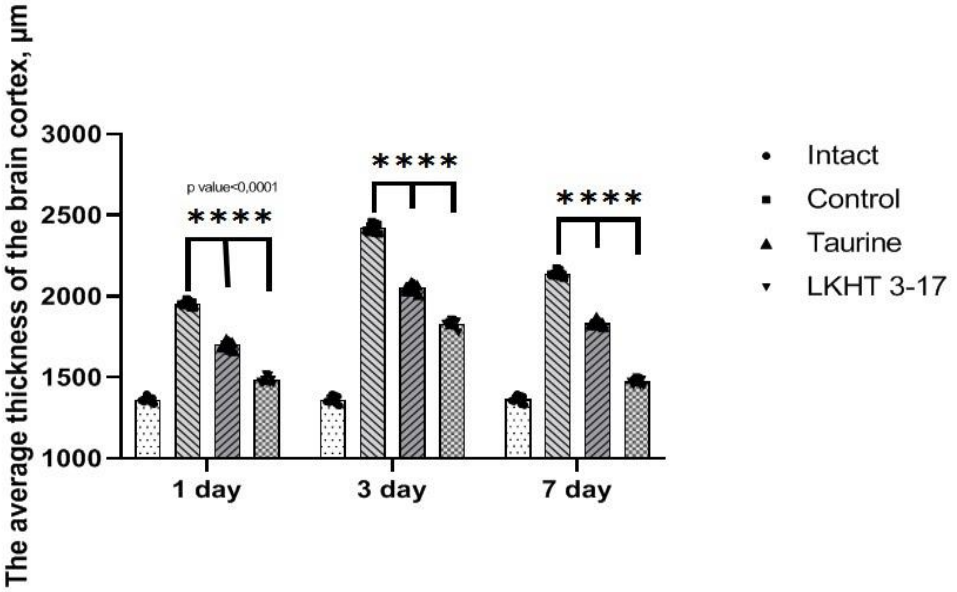

Figure 3. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the average thickness of the brain cortex (µm) in experimental hemorrhagic stroke on the 1st, 3rd, and 7th days.
Note: Dynamics of the average thickness of the brain cortex in the studied groups by assessing the severity in experimental hemorrhagic stroke.

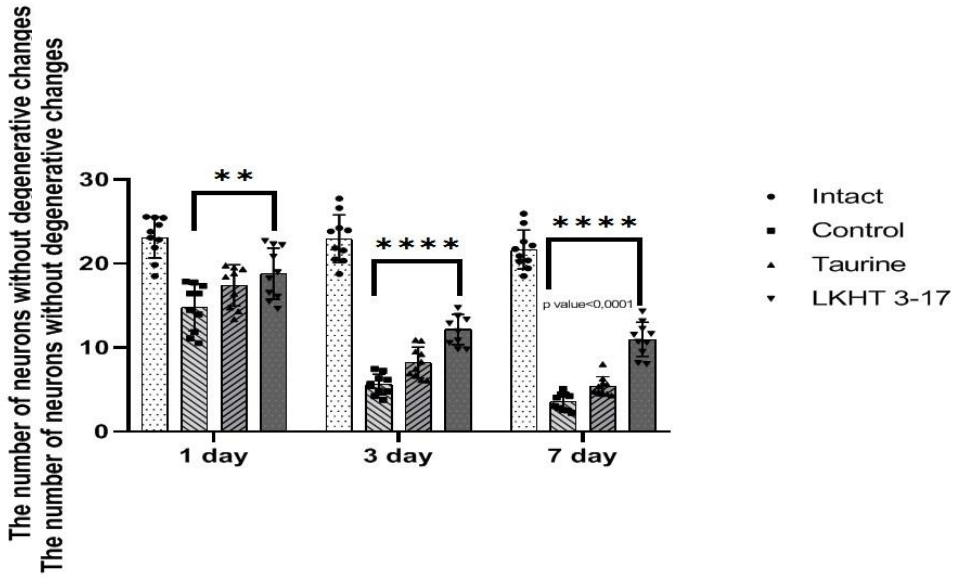

Figure 4. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the number of neurons without degenerative changes in experimental hemorrhagic stroke on the 1st, 3rd, and 7th days.
Note: Dynamics of the number of neurons without degenerative changes in the studied groups by assessing the severity in experimental hemorrhagic stroke.

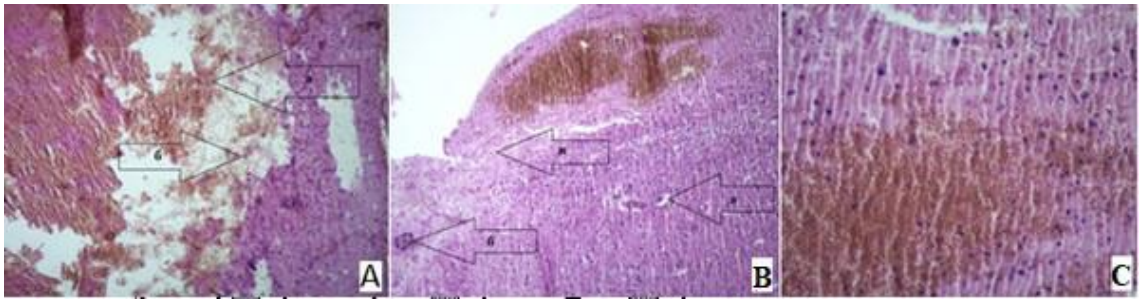

Figure 5. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of brain morphological changes in experimental hemorrhagic stroke on the 1st day. Photomicrography of the rat's brain section on the 1st day after hemorrhagic stroke simulation, stained with hematoxylin and eosin, magnification ×100. **A, B –** control group, **C –** LKHT 3-17. **A-** The area of hemorrhage, where, a - red blood cells, b - segmental leukocytes. **B-** The peripheral zone of the stroke, where, a-perivascular edema; b– infiltration of the vein wall by leukocytes, **C-** areas of the brain tissue destruction.

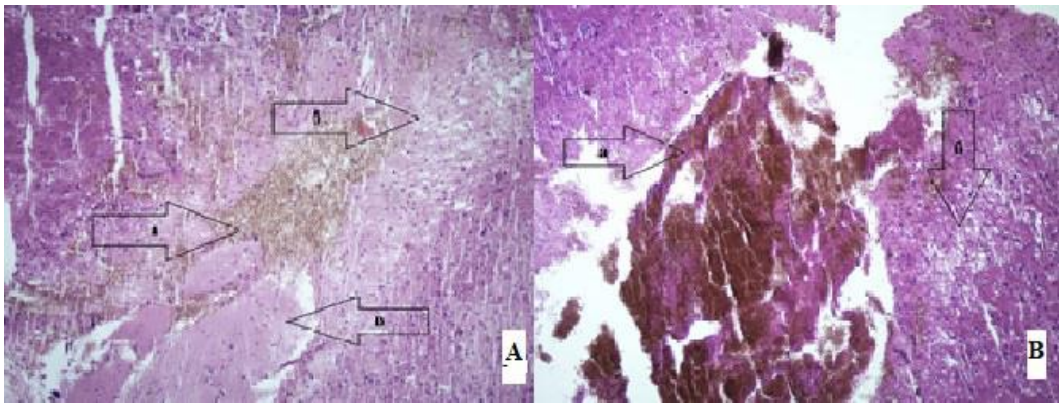

Figure 6. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of brain morphological changes in experimental hemorrhagic stroke on the 3rd day. Photomicrography of the rat's brain section on the 3rd day after hemorrhagic stroke simulation, stained with hematoxylin and eosin, magnification ×100. a - the area of hemorrhage; b - brain swelling; c - infiltration of the vein wall with white blood cells, c-areas of destruction (necrosis) of the brain tissue with the nests of white blood cells. B- rat's brain section on the 3rd day after LKHT 3-17 administration, stained with hematoxylin and eosin, magnification ×100.a -the area of hemorrhage, b- brain swelling.

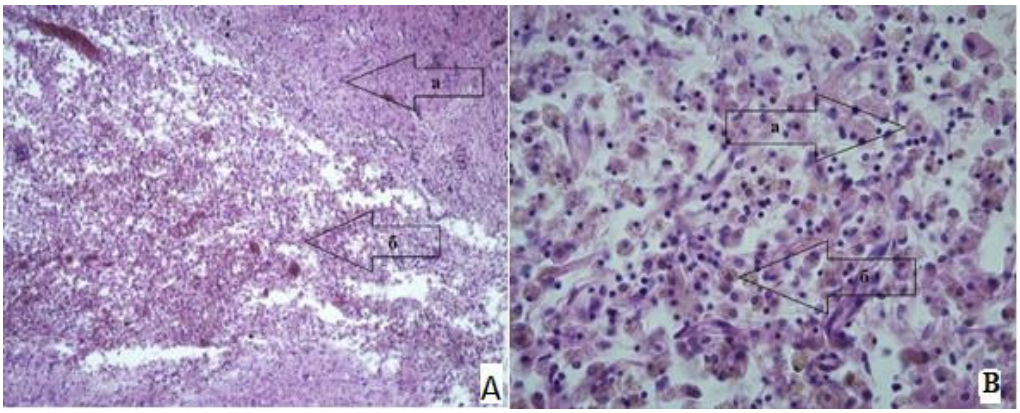

Figure 7. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of brain morphological changes in experimental hemorrhagic stroke on the 7th day. Photomicrography of the rat's brain section on the 7th day after hemorrhagic stroke simulation, stained with hematoxylin and eosin. **A –** Control group, magnification ×100. B - LKHT 3-17 group, magnification ×400. **A -** Where, a - zone of perifocal hemorrhage, b - zone of hemorrhage with the nests of macrophages, hemosiderophages. B - where, a-macrophage; b – hemosiderophage.

Therefore, the obtained results indicate that the most prominent cerebroprotective effect in the correction of hemorrhagic stroke in Wistar rats, comparable to the reference drug taurine at the dose of 50 mg/kg, has the substance LKHT 3-17 at the dose of 150 mg/kg. That is expressed in reducing lethality, minimizing the neurological deficits, increasing the locomotor activity, reducing the volume, and accelerating the processes of rehabilitation of hemorrhagic focus.

4. Discussion

Several studies were conducted on the optimal management of hemorrhagic stroke- antihypertensive treatment for acute cerebral hemorrhage, reduction of blood pressure in acute cerebral hemorrhage, factor VIIa for the treatment of acute hemorrhagic stroke, and surgery (24). Primary trauma occurs due to compression by the hematoma and increased intracranial pressure (25). The size of the hematoma usually increases from 3 to 12 hours. In a third of cases, the hematoma enlarges for 3 hours. Perihematomal edema increases 24 hours later, peaks after about 5-6 days, and lasts up to 14 days. There is a hypoperfusion zone around the hematoma. The factors that worsen the pathological process include increased hematoma, intraventricular hemorrhage, perihematomal edema, and inflammation. Cerebellar hemorrhage causes hydrocephalus as a result of compressing the fourth ventricle in the early stages (26).

Taurine can effectively prevent glutamate-induced neuronal damage in cultured neurons. In addition, taurine can protect against H_2O_2-induced cell damage in PC12 cell culture due to the reduction of H_2O_2-induced ER stress. It is generally believed that the neuroprotective effects of taurine are due to its role in reducing the intracellular concentration of free Ca^{2+}, $[Ca^{2+}]i$, and its antioxidant stress capacity (27, 28).

Magnesium performs several regulatory functions in neuronal and neuromuscular synapses as an endogenous calcium antagonist. It blocks the flow of calcium into the presynaptic terminals and prevents excessive release of acetylcholine and stimulation of the neuromuscular junction. It also has an inhibitory effect on the postsynaptic membrane through the potential-dependent blockade of N-methyl-D-aspartate receptors (NMDA). This acts as an NMDA receptor antagonist which underlies one of the main proposed mechanisms of magnesium neuroprotection (29).

Studies of hemorrhagic strokes mainly focus on the use of magnesium sulfate in aneurysmal subarachnoid hemorrhage. The condition of approximately one-third of survivors worsens 3-14 days after bleeding as a result of delayed cerebral ischemia. The etiology of this process is probably multifactorial, including oxidative stress, vasoconstriction, inflammation, and depression spreading through the cortices (30).

The fields of application of succinic acid are very diverse which include cardiology, neurology, endocrinology, toxicology and narcology, infectious diseases, pediatrics, and rehabilitation medicine. These medications are used quite successfully in surgery, pulmonology, hematology, dermatology, obstetrics, and gynecology. Such functional diversity of succinic acid is due to the direct participation of succinate in the processes of cellular respiration and oxidative phosphorylation in mitochondria.

The prerequisite for the use of succinic acid medications in cardiology was, first of all, maintaining the energy-synthesizing ability of cells in hypoxic conditions. Based on the key role of atherosclerosis in the pathogenesis of cardiovascular diseases, the most radical method of treatment is restoring impaired blood supply (31, 32).

The present study indicates that the taurine derivative of magnesium-bis-(2-aminoethanesulfonic)-butadioate, which is a combination of amino acid, magnesium ion, and succinic acid reduces the neurological deficits, lethality, and increases the locomotor and exploratory behavior in experimental hemorrhagic stroke in rats. The effect of the studied medication on the dynamics of molecular pathophysiological mechanisms occurring in the cell requires further research.

Authors' Contribution

Study concept and design: N. I. N. and E. A. P.
Acquisition of data: N. I. N. and V. V. P.
Analysis and interpretation of data: I. V. P. and T. G. P.
Drafting of the manuscript: N. B. L.
Critical revision of the manuscript for important intellectual content: M. I. I. and S. N. M. K.
Statistical analysis: A. V. N. and V. I. S.
Administrative, technical, and material support: N. I. N., E. A. P. and Y. A. H.

Ethics

All procedures performed in this study involving animal participants were in accordance with the Russian Scientific Center for the Safety of Bioactive Substances (StarayaKupavna, Russia) and provided for experiments by Sofia Ya. Skachilovaunder laboratory code LKHT 3-17.

Conflict of Interest

The authors declare that they have no conflict of interest.

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