Evaluation of the Neuroprotective Effect of Magnesium-Bis-(2-Aminoethanesulfonic)-Butadioate in Simulated Ischemic Stroke in Rats


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Abstract

Sudden loss of blood flow to an area of the brain causes ischemic stroke, which leads to a loss of nerve function in the brain. The brain tissue leads to the death of brain cells in less than a few minutes due to the lack of oxygen and nutrients. This study aimed to evaluate the effectiveness of pharmacological correction of the consequences of ischemic stroke with a new derivative of taurine magnesium-bis-(2-aminoethanesulfonic)-butadioate under laboratory code LKHT 3-17 in rats. The ischemic stroke was simulated by electrocoagulation of the right middle cerebral artery. The assessment of lethality, neurological status, locomotor, and exploratory behavior, and morphological pattern of the brain damage was carried out on the 1st, 3rd, and 7th day after the pathology simulation. Neurological deficit was determined by the McGrow stroke index scale. The locomotor and exploratory behavior was evaluated using the Acti-track software and hardware complex. When assessing the morphological changes in the brain, attention was paid to two criteria: the average thickness of the brain cortex and the number of neurons without degenerative changes. The substances were administered 60 minutes before the start of surgery. The animals were divided into the following groups: Intact group (n=20); ischemic stroke simulation without pharmacological correction (n=50); a group with correction of the ischemic stroke with taurine at the dose of 50 mg/kg (n=50); a group with correction of ischemic stroke with magnesium-bis-(2-aminoethanesulfonic)-butadioate (LKHT 3-17) at the dose of 150 mg/kg (n=50). LHT 3-17 (150 mg/kg) and taurine (50 mg/kg) reduced lethality by 1.55 and 1.47 times respectively on the 7th day after stroke compared to the control group.
In parallel, an effective correction of neurological deficit was found for LKHT 3-17 and taurine to 4.0±0.8 and 7.6±0.9 respectively on the 3rd day in contrast to the control of 8.1±0.8 points. The locomotor and exploratory behavior was most significantly different on the 1st and 7th days and was accompanied by a significant increase in the speed of movement under the influence of LKHT 3-17 to 20 and 20 conventional units (CU) compared to the control of 7 and 5 cu. On the 1st day, the thickness of the cortex was 1877.3±43.3 µm in the control group, and 1531.8±39.1 µm in LKHT 3-17 group. The number of neurons without neurodegenerative changes prevailed in the group administered with LHT 3-17 (19.3±4.3), and the lowest number was observed in the group without pharmacological correction of the pathology (14.3±3.7). LKHT 3-17 at a dose of 150 mg/kg is more effective than taurine 50 mg/kg in protecting nerve activity in experimental ischemic stroke and reduces lethality, minimizes nerve defects, reduces volume, and accelerates the process of tissue repair. Seeing helps in stroke and activates the regenerative processes.

**Keywords:** Ischemic Stroke, Taurine, Neuroprotection

### 1. Introduction

Prevention and treatment of cerebrovascular injuries are one of the priorities of every country. Stroke is the leading cause of death in Russia. The number of cerebrovascular diseases is increasing every year. High mortality due to stroke, cerebral ischemia, etc. has caused many problems such as vision problems, such as blindness in one eye or double vision, weakness or paralysis in your limbs, which may be on one or both sides, depending on the affected artery, dizziness and vertigo (1). Decreased perfusion can lead to stroke and lead to permanent nerve failure or death. Ischemic stroke accounts for 85% of strokes. Ischemic cascade is caused by a decrease in cerebral perfusion, which causes a reversible ischemic network in the irreversible area of the stroke (2).

Recently, a large number of studies have been devoted to the study of various pharmacological agents with antioxidant activity, such as platinum nanoparticles, for the correction of ischemic damage to the central and peripheral nervous system, which emphasizes the relevance of the problem (3-7). Preclinical studies of new molecular compounds should include molecular docking, molecular screening, assessment of specific activity, and morphological changes to find proper treatment for stroke treatment (8-11).

Taurine is a beta-amino acid that is found in very high concentrations in most cells, especially in excitable tissues (8). This amino acid plays a significant role in most physiological activities including membrane stabilization, antioxidant activity, detoxification, altered immune response, calcium transfer, cardiac muscle contractions, corneal development, bile acid metabolism, osmotic regulation, and endocrine functions. Interestingly, taurine is also released by brain slices in response to
stimulation of the glutamate receptor (10). The release of taurine decreases the side effects of glutamate toxicity by reducing \([\text{Ca}^{2+}]\), increasing the Bcl-2/Bad ratio, and suppressing ER stress (10). In addition, taurine reduces cell swelling in sections of rat’s cerebral cortex subjected to hypoxic reoxygenation (5, 6). The functions of taurine in the body include maintaining hydration as well as the balance of electrolytes in the body's cells, helping to digest food by forming bile salts, balancing the transport of minerals into cells, helping the main activities of the eye and central nervous system, and it also plays an antioxidant role and its effect on regulating the immune system (12). Two organs, the heart, and the brain are known to produce their taurine, but in very limited quantities. In humans, high concentrations of taurine can be found in plasma, bile, saliva, and heart tissue. Taurine has a positive effect on liver function, gastrointestinal damage, kidney disease, diabetes, and cardiovascular disease (13).

The studied substance magnesium-bis-(2-aminoethanesulfonic)-butadioate under laboratory code LKHT 3-17 was synthesized in Russian Scientific Center for the Safety of Bioactive Substances (Staraya Kupavna, Russia) and provided for experiments by Sofia Ya. Skachilova. This study aims to evaluate the effectiveness of pharmacological correction of the consequences of ischemic stroke by the new taurine derivative magnesium-bis-(2-aminoethanesulfonic)-butadioate under laboratory code LKHT 3-17 in rats.

2. Materials and Methods

2.1. Animals

The experiments were performed in 170 white male and female Wistar rats weighing 250 ± 25 g without external signs of the disease, who were put into quarantine for 10-14 days. The animals were kept in accordance with the rules of laboratory practice for conducting preclinical studies in the Russian Federation (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 were provided with a standard laboratory feeding and water.

2.2. Research design

LHT 3-17 at the dose of 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 the ischemic stroke without pharmacological correction (n=50)
3. Correction of the ischemic stroke with taurine at the dose of 50 mg/kg (n=50)
4. Correction of the ischemic stroke with magnesium-bis-(2-aminoethanesulfonic)-butadioate at the dose of 150 mg/kg (n=50).

2.3. Simulation of the ischemic stroke
We performed electrocoagulation of the right middle cerebral artery, immediately at the place of its origination from the cerebral arterial circle (14). The right middle cerebral artery (MSA) was exposed by the maxillary cutdown approach and coagulated using an electrocoagulator (MVS 100). The effectiveness was evaluated by the color change of the MSA distal to the site of coagulation.
Assessment of the lethality, neurological deficit, locomotor activity and morphological examination were carried out on the 1st, 3rd, and 7th days after the simulation of the ischemic stroke.

2.4. Assessment of the neurological status of animals
Neurological deficit in the animals with pathology 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 noted: with mild symptoms (up to 2.5 points on the stroke index scale) – sluggishness of movements, weakness of the limbs, unilateral 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 (15).

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

2.6. Assessment of brain changes by histological examination
For morphological evaluation, after 24-hour fixation in 10% neutral formalin, 2 pieces of the brain with a thickness of 0.2-0.3 cm were examined. The section was made in the frontal plane through the entire brain of each animal. The specimens 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 Leica EG 1150 H, Leica RM 2245, and Leica autostainer XL devices. Two criteria were statistically evaluated: the average thickness of the brain 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 the standard error of the mean (m) were calculated. The outliers in each time point were identified using Grubb’s test. If for any sample, the value of Z was greater than the critical value for the given number of measurements N, this sample was excluded from further calculations. In cases of non-normal distribution, the median (Me) and quartile range (QR) were calculated. The inter-group differences were analyzed by parametric (Student’s t-test) or nonparametric (Mann-Whitney 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 10.0 software.

3. Results

3.1. Lethality

It was found that on the first day after ischemic stroke simulation, the lethality rate in the control group was 55%. By the third day, the lethality rate increased to 70%, and by the 7th day, the lethality rate increased to 84%. In the group administered with LKHT 3-17 at the dose of 150 mg/kg, the lethality rate 24 hours after the pathology simulation was 30%. By the third day, the lethality rate increased to 34%, and on the 7th day it was 50%. In the comparison group of taurine at the equimolar dose 50 mg/kg, the lethality rate was 64%, which corresponds to the values in the control group. By the third day, the lethality rate was 64% from the input animal number, and by the 7th day it increased to 60% (Figure 1).
Figure 1. The effect of LHT 3-17 150 mg/kg and taurine 50 mg/kg on the lethality dynamic in experimental ischemic stroke

Note: Effect of the studied drugs on rat survival in simulated ischemic stroke in the experiment.

3.2. Assessment of the neurological status of the animals

According to the data obtained in the experimental groups with simulated ischemic stroke, LKHT 3-17 at the dose of 150 mg/kg significantly reduces the severity of neurological disorders not only in comparison with the control group, but also in comparison with the reference compound - taurine at the dose of 50 mg/kg (Table 1).
Table 1. 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 ischemic stroke (M±m)

<table>
<thead>
<tr>
<th></th>
<th>Intact group</th>
<th>Control</th>
<th>Taurine</th>
<th>LKHT 3-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st day</td>
<td>0</td>
<td>7.9±0.8</td>
<td>7.8±0.9</td>
<td>5.2±0.8*</td>
</tr>
<tr>
<td>3rd day</td>
<td>0</td>
<td>8.1±0.8</td>
<td>7.6±0.9</td>
<td>4.0±0.8*</td>
</tr>
<tr>
<td>7th day</td>
<td>0</td>
<td>5.0±0.5</td>
<td>3.5±1.0*</td>
<td>2.0±1.0*</td>
</tr>
</tbody>
</table>

Note: * p<0.0001 - the differences are statistically significant in comparison with the control group of the corresponding time point;

3.3. Evaluation of the locomotor and exploratory behavior

Assessing the locomotor activity of the animals in the IR Actimeter test found that the pathology simulation causes a significant decrease in locomotor activity on the 1st day, followed by recovery by the 7th day (Figures 1,2,3,4).

In the group administered with LKHT 3-17 the number of total activity was higher on day 1st by 1.6, on 3rd by 1.67 on day 7th – by 4.49 times compared to the control group (Figure 2).

Figure 2. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the total activity (cu) of the animals with experimental ischemic stroke on the 1st, 3rd and 7th days

Note: The effect of the studied drugs on the total activity in the actimetry test.

In the group administered with LKHT 3-17 the number of stereotypes was higher on day 1st by 3.2, on 3rd-by 1.5 on day 7th – by 2.25 times compared to the control group (Figure 3).
Figure 3. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the stereotypies (cu) of the animals with experimental ischemic stroke on the 1st, 3rd and 7th days.

Note: The effect of the studied drugs on the motor stereotypy in the actimetry test.

In the group administered with LKHT 3-17 the number of general locomotor activity was higher on day 1st by 2.75, on 3rd-by 1.9 on day 7th – by 2.3 times compared to the control group (Figure 4).

Figure 4. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the general locomotor activity (cu) of the animals with experimental ischemic stroke on the 1st, 3rd and 7th days.

Note: The effect of the studied drugs on the general locomotor activity in the actimetry test.
In the group administered with LKHT 3-17 the number of the distance covered activity was higher on day 1st by 2.8, on 3rd by 1.69 on day 7th – by 2.06 times compared to the control group (Figure 5).

- Intact
- Control
- Taurine
- LKHT 3-17

**Figure 5.** The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of the distance covered (cu) of the animals with experimental ischemic stroke on the 1st, 3rd and 7th days.

Note: The effect of the studied drugs on the total distance in the actimetry test.

### 3.4. Evaluation of brain changes by histological examination

According to the data presented in table 3, 24 hours after the simulation of the ischemic stroke, the greatest thickness of the brain cortex was observed in the control group (1877.3±43.3), and the least - in the group of experimental compound (1531.8±39.1). The number of neurons without neurodegenerative changes prevailed in the group of the studied compound (19.3±4.3), and the lowest number was observed in the group without pharmacological correction of the pathology (14.3±3.7) (Table 2). Dynamic changes in the studied morphological parameters had the following features: thickening of the cerebral cortex in the control group was noted by the third day, later its dynamic decline was observed, which was also noted in the group of the studied compound.

### Table 2. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics of brain morphological changes in experimental ischemic stroke on the 1st, 3rd and 7th day (M±m)

<table>
<thead>
<tr>
<th>Day</th>
<th>Sings</th>
<th>Intact group</th>
<th>Control</th>
<th>Taurine</th>
<th>LKHT 3-17</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The average thickness of the brain cortex, µm</td>
<td>The number of neurons without degenerative changes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---------------------------------------------</td>
<td>-------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st day</td>
<td>1361.8±36.9</td>
<td>23.3±4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1877.3±43.3</td>
<td>14.3±3.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1778.1±42.16*</td>
<td>15.7±3.9**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1531.8±39.1*</td>
<td>19.3±4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd day</td>
<td>1361.8±36.9</td>
<td>23.3±4.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2128.2±46.1</td>
<td>6.3±2.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1887.3±43.44*</td>
<td>10.33±3.2</td>
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</tr>
<tr>
<td></td>
<td>1684.8±41.04*</td>
<td>13.3±3.6*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7th day</td>
<td>1361.8±36.9</td>
<td>23.3±4.8</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>1979.6±44.4</td>
<td>4.7±2.16</td>
<td></td>
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<td></td>
<td>1700.1±41.23*</td>
<td>9.3±3.04</td>
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<tr>
<td></td>
<td>1552.1±39.3*</td>
<td>12.7±3.5*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *p<0.0001 p<0.005 - the differences are statistically significant when compared with the control group of animals at the corresponding time point.

Morphological pattern in the studied groups A control, B - LKHT 3-17. Photomicrography of the rat’s brain section on the 1st day after right middle cerebral artery ischemic stroke simulation, stained with hematoxylin and eosin. A - magnification ×100, where a - the central area of the stroke; b - the peripheral area of the stroke (penumbral area)
Photomicrography of the rat’s brain section on the 3rd day after right middle cerebral artery ischemic stroke simulation, A - control, B – after administration of LKHT 3-17, stained with hematoxylin and eosin, magnification ×100. A: a - the central area of the stroke with a pronounced inflammatory reaction; b - the peripheral area of the stroke (penumbral area) with vascular proliferation, accumulation of macrophages and glial cells. B: a - the infarction area with an accumulation of white blood cells and macrophages; b – vascular proliferation; c - penumbral area with the beginning of the formation of an immature gliomezodermal scar.

Photomicrography of the rat’s brain section on the 7th day after right middle cerebral artery ischemic stroke simulation, A - control, magnification ×200. B - LKHT 3-17, magnification ×100. Staining with hematoxylin and eosin. A: a - the central area of the stroke with the accumulation of segmental leukocytes and CNS-associated macrophages; b - the (penumbral area with perivascular, pericellular edema, a pronounced glial reaction in the form of neuronophagy. B: - the area of infarction with the accumulation of macrophages, the formation of gliomezodermal scar.

Thus, the obtained results indicate that the most pronounced cerebroprotective effect in the correction of ischemic stroke in Wistar rats, comparable to the reference compound 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 and reducing the volume and accelerating the processes of rehabilitation of ischemic focus.

4. Discussion
Magnesium-bis-(2-aminoethanesulfonic)-butadioate is a combination of the amino acid, a magnesium ion, and succinic acid. Taurine has multiple points of influence on pathophysiological processes in the pathology development of the central nervous system. The presence of magnesium ions, as well as succinic acid, allows expanding the impact on various mechanisms of the pathological process in the CNS tissues.

Possible mechanisms of the protective effects of magnesium in various pathological conditions may include its antioxidant and anti-inflammatory effects (16, 17). Moreover, it has been suggested that magnesium may provide protection against edema by suppressing the activity of aquaporin-4 in brain tissue after traumatic brain injury (18). The beneficial effects of magnesium in reducing brain edema may also be related to its role as a physiological calcium antagonist, blocking the NMDA glutamate receptor (19) and reducing oxidative stress (17).

Other effects of taurine, such as antioxidant, anti-inflammatory, or osmoregulatory, may also contribute to its neuroprotective action (20-22) against ischemic stroke. In the presence of high calcium content in mitochondria, the physiological production of reactive oxygen species [(ROS: such as superoxide anion (O²⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (OH))] and nitric
oxide NO, becomes pathological due to the imbalance of their production compared to their degradation. This pathological condition, commonly called oxidative stress, is observed in ischemic stroke (23).

The main pathophysiological mechanisms involved in ischemic stroke are excitotoxicity of glutamate, calcium imbalance, and oxidative stress, which individually or collectively leads to cell death. Thus, the role of taurine as an inducer of inhibitory neurotransmission, an antioxidant, a neuromodulator, a regulator of calcium homeostasis, and a neuroprotector potentially makes it an ideal therapeutic agent for ischemic stroke.

Edema is one of the conditions that occur after an ischemic stroke of the brain. This is caused by the accumulation of intracellular Na⁺ and Cl⁻, which leads to osmotic influx of water. The edema then increases the release of taurine; initially, the release is exocytosis and Ca²⁺-dependent, then through the reverse Na+/Cl-dependent mode, because the ischemic stroke persists, the release occurs through the volume-activated Cl⁻ channels and finally by diffusion through the permeable plasma membrane (24). This regulates the cell volume to preventing its death due to necrotic swelling. It was shown that taurine significantly reduced the cell swelling in the cortical sections of the rat’s brain after exposure to oxygen-glucose deprivation and reoxygenation (19, 25).

In addition to antihypoxic and antioxidant effects, medications of the succinic acid have nootropic, anticonvulsant, and anxiolytic effects (25). Drugs of this group modulate the activity of cell membrane enzymes (Ca²⁺-independent phosphodiesterase, adenylate cyclase, acetylcholinesterase), receptor complexes (benzodiazepine, GABA, acetylcholine), promoting their binding to ligands, preserving the structural and functional organization of biomembranes, neurotransmitter transport, and improving synaptic transmission; they increase the concentration of dopamine in the brain, enhance the compensatory activation of aerobic glycolysis. In the experiment, the use of the succinic acid in acute cerebral ischemia in laboratory rats led to a decrease in the neuron destruction, a decrease in the concentration of lipid peroxidation products, ammonium ions, α-alanine, normalization of the contingency coefficient of oxidative phosphorylation, and, ultimately, to an increase in survival (26). Thus, the potentiation of the effects in the studied taurine derivative mediates the need for further study of this drug in order to pharmacological correction the pathology of the central nervous system. In conclusion, LKHT 3-17 at the dose of 150 mg/kg exceeds the equimolar dose of taurine 50 mg/kg in neuroprotective activity in the experimental ischemic stroke. That is expressed in reducing lethality, minimizing the neurological deficit, reducing the volume and accelerating the processes of rehabilitation of the stroke focus, activating regenerative processes.

References


