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

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Abstract

Stroke or ischemia is caused by a partial or complete reduction in blood flow to the brain. Ischemia may be caused by cardiac arrest or by blockage of a specific blood vessel. Nutritional factors such as antioxidants and healthy eating patterns are important variables for stroke. Molecular composition properties, which include molecular binding and molecular screening, can be used to evaluate the specific activity and morphological changes. The aim of this study was to evaluate the effectiveness of pharmacological correction of the consequences of a hemorrhagic stroke with a new derivative of taurine magnesium-bis-(2-aminoethanesulfonic)-butadioate in rats. The animals (n=170) were divided into four groups as follows: 1) Control group (n=20); 2) Group 2 underwent the 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). A hemorrhagic stroke was induced by introducing autologous blood into the parietal lobe of the right hemisphere of the brain. 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 days after the pathology simulation. Neurological deficit in animals 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 in microns 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 the control (p<0.05). In parallel, an effective correction of neurological deficit was found for 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 most 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 in the control group, and 1491.0±38.61 μm in LKHT 3-17 group. 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)-butadiolate, which is a combination of the amino acid, a 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 drug on the dynamics of molecular pathophysiological mechanisms occurring in the cell requires additional research.

**Keywords:** Hemorrhagic stroke, Neuroprotection, Taurine

1. Introduction

Hemorrhagic stroke is a stroke caused by a brain hemorrhage that occurs with a ruptured cerebral artery. It accounts for the percentage of deaths due to stroke (1). Hemorrhage occurs due to rupture of cerebral arteries, cerebral aneurysms, and weak-walled rupture can cause hemorrhagic stroke. Hemorrhagic stroke is divided into two types included subarachnoid or subarachnoid ingestion and bleeding. It is called intracerebral or intracerebral (2). The Russian Stroke Association reports that 40,000 intracerebral hemorrhages are registered in Russia annually. The relevance of the problem of cerebrovascular pathology is currently important due to the high lethality and severe disability of patients. The problems of new drug development for the treatment of this pathology come to the fore. The relevance of cerebroprotective drugs is important not only in neurology, neurosurgery, but also in pediatrics and ophthalmology. Recently, a large number of studies, devoted to various types of neuroprotective drugs, have been published. That clearly demonstrates 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 (8, 9). Thus, the amino acid taurine is widely used in ophthalmological practice, but in the Russian Federation, in neurology and neurosurgery, it has not found its application. 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, and maintaining intracellular homeostasis (10). In foreign sources, the pharmacological effect of this substance is noted in the correction of cerebrovascular pathology (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 and 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 urinary taurine excretion groups, taurine replacement, and body mass index, blood pressure, cholesterol levels, and stroke mortality rates (16-20).

In simulated stroke in rats, taurine reduced glutamate-mediated toxicity by reducing oxidative stress and [Ca^{2+}] overload. 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). There is a lot of evidence 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).

This study aimed to evaluate the effectiveness of 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. Materials and methods

2.1. Animals

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. The experiments were performed in 170 white male 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 (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 hemorrhagic stroke without pharmacological correction (n=50)
3. Correction of the hemorrhagic stroke with taurine at the dose of 50 mg/kg (n=50)
4. Correction of the hemorrhagic stroke with magnesium-bis-(2-aminoethanesulfonic)-butadioate at the dose of 150 mg/kg (n=50).

2.3. Simulation of the hemorrhagic stroke
Simulation of the hemorrhagic stroke was performed according to the previously described method in the Patent №2721289 (13).
After the proper and deep anesthesia, autologous blood was sampled from the tail vein, in a volume of 0.1 ml/100 g.
Using dental boron (NTI (Germany)) a burr hole about 3 mm of the diameter was made in the rats' skull. Cerebral tissue are decomposed by rotary motions of mandren-knife in three turns clockwise direction and then counterclockwise. After that, the mandren-knife was removed and autologous blood was injected under pressure of 110 mm Hg.
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 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 were noted: with mild symptoms (up to 2.5 points on the stroke index scale) – sluggishness of movements, weakness of the limbs, unilateral hemiptosis, 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 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 hemorrhagic stroke simulation, the lethality rate in the control group was 44% (Figure 1). By the third day, the lethality rate increased to 68%, and by the 7th day, the lethality rate increased to 78%. In the group administered with LKHT 3-17 (150 mg/kg), the lethality rate 24 hours after the pathology simulation was 8%. By the 3rd, the lethality rate increased to 40%, and on the 7th day it was 44%. In the comparison group of taurine at the equimolar dose (50 mg/kg), the lethality rate on the first day was 14%. By the third day, the lethality rate increased to 50%, and by the 7th day it was 60%.
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: Effect of the studied drugs on rat survival in simulated hemorrhagic 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 hemorrhagic stroke, LKHT 3-17 (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 drug - taurine (50 mg/kg) (Figure 2).
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 assessment of the severity of neurological deficit in meningitis, meningoencephalitis (by the average score in the group).

3.3. Evaluation of the locomotor and exploratory behavior

Assessing the locomotor activity of the animals in the IR Actimeter test found that the activity of the animals administered with the test drug significantly increased throughout the experiment in comparison with the group without pharmacological correction of hemorrhagic stroke (Table 1).
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)

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

Note: *** p<0.0001 * p<0.0015 - the differences are statistically significant in comparison with the control group of the corresponding time point

3.4. Evaluation of brain changes by histological examination

According to the data presented in table 2, 24 hours after the simulation of the hemorrhagic stroke, the greatest thickness of the brain cortex was observed in the animals without pharmacological correction (1943.7±44.08), and the least - in the group of experimental drug (1491.0±38.61) (Figure 3 and 5). The most number of neurons without degenerative changes was observed in the LKHT 3-17 group (18.7±4.32) in comparison with the control group (14.3±3.78) (Figure 5, 6, and 7). At further control points, the following dynamic of morphological changes were observed: the thickness of the brain cortex in the control group reached its maximum value on the third day (2424.0±49.2) (Figure 4 and 6).
Figure 3. The effect of LKHT 3-17 150 mg/kg and taurine 50 mg/kg on the dynamics the average thickness of the brain cortex, μm in experimental hemorrhagic stroke on the 1st, 3rd and 7th day. 

Note: Dynamics of the average thickness of the brain cortex in the studied groups on the sale of assessment of 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 the number of neurons without degenerative changes in experimental hemorrhagic stroke on the 1st, 3rd and 7th day. 

Note: Dynamics of the number of neurons without degenerative changes in the studied groups on the sale of assessment of 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 1\textsuperscript{st} day. Photomicrography of the rat’s brain section on the 1\textsuperscript{st} 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 3\textsuperscript{rd} day. Photomicrography of the rat’s brain section on the 3\textsuperscript{rd} 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 3\textsuperscript{rd} 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 x100. B - LKHT 3-17 group, magnification x400. A - where, a - zone of perifocal hemorrhage, b - zone of hemorrhage with the nests of macrophages, hemosiderophages., B - where, a -macrophage; b – hemosiderophage.

Thus, the obtained results indicate that the most pronounced 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 and reducing the volume and accelerating the processes of rehabilitation of hemorrhagic focus.

4. Discussion
There are many studies on the optimal management of hemorrhagic stroke - antihypertensive treatment for acute cerebral hemorrhage, study of intensive lowering 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). Usually, the hematoma increases in size from 3 to 12 hours. In a third of cases the hematoma increases for 3 hours. Perihematoma edema increases 24 hours after, peaks about 5-6 days after and lasts up to 14 days. There is a hypoperfusion zone around the hematoma. The factors that cause the worsening of the pathological process are an increase in hematoma, intraventricular hemorrhage, perihematoma edema and inflammation. Cerebellar hemorrhage causes hydrocephalus as a result of compression of the fourth ventricle at an early stage (26).

Taurine can effectively prevent glutamate-induced neuronal damage in cultured neurons. In addition, taurine can protect against cell damage caused by H$_2$O$_2$ in PC12 cell culture due to reducing ER stress.
caused by H$_2$O$_2$. 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).

As an endogenous calcium antagonist, magnesium performs a number of regulatory functions in neuronal and neuromuscular synapses. It blocks the flow of calcium into the presynaptic terminals, preventing excessive release of acetylcholine and stimulation of the neuromuscular junction. It also has an inhibitory effect on the postsynaptic membrane via the potential-dependent blockade of N-methyl-D-aspartate receptors (NMDA). This action as an NMDA receptor antagonist 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. 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 and include cardiology, neurology, endocrinology, toxicology and narcology, infectious diseases, pediatrics, and rehabilitation medicine. These drugs are used quite successfully in surgery, pulmonology, hematology, dermatology, obstetrics and gynecology. Such a variety of applications of succinic acid is due to the direct participation of succinate in the processes of cell respiration and oxidative phosphorylation in mitochondria.

The prerequisite for the use of succinic acid medications in cardiology was, first of all, the ability of succinate to maintain 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 the restoration of impaired blood supply (31, 32).

In the presented study, it was shown that the taurine derivative magnesium-bis-(2-aminoethanesulfonic)-butadioate, which is a combination of the amino acid, a 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 drug on the dynamics of molecular pathophysiological mechanisms occurring in the cell requires additional research.

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