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THE SEARCH FOR NEUROPROTECTIVE COMPOUNDS AMONG NEW ETHYLTHIADIAZOLE DERIVATIVES

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The aim of the study is to search compounds with neuroprotective properties among new ethylthiadiazole derivatives in simulated traumatic brain injury.
Materials and methods. The experiment was carried out on 78 white male rats 270±20 g line "Wistar" 5–6 months of age and 120 outbred sexually mature mice weighing 20±2 grams. The article describes the search for compounds with neuroprotective properties among new ethylthiadiazole derivatives under the codes LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 in experimental traumatic brain injury in rats. Acute toxicity of the compounds was studied. Pharmacological screening was performed using behavioral and neurological research methods. The McGraw stroke score scale modified by I.V. Gannushkina and the mNSS psychometric scale were used in the study. The open field and Rota-rod tests were used to assess the behavioral status of the animals.
Results. The compound-LKHT 12–18 at a dose of 50 mg/kg was detected as a leader. In pharmacological correction of pathology, this compound had the lowest percentage of fatality among the studied compounds (8%), the severity of neurological deficit was significantly reduced, the lowest scores and a higher level of motor activity of the limbs were registered. The number of rearing in the group of animals receiving the compound LKHT 12–18 at the dose of 50 mg/kg increased by 1.5 times, statistically significant ($p<0.05$) in comparison with the control group. Based on the results of the "Rota-rod" test, the total time of holding animals on the rod for 3 attempts was statistically significantly different in the groups administered with LKHT 12–18 derivatives (1.5 times longer) at the dose of 50 mg/kg compared with the control ($p<0.05$).
Conclusion. Based on the results obtained in this study, it is planned to study in more detail the compound LKHT 12–18 at the dose of 50 mg/kg
Keywords: traumatic brain injury, ethylthiadiazole derivatives, neuroprotection
Abbreviations: TBI – traumatic brain injury; ATP – adenosine triphosphate; DAI – diffuse axonal injury; tSAH – traumatic subarachnoid hemorrhage; BBB – blood-brain barrier; LP – latency period

ИССЛЕДОВАНИЕ НЕЙРОПРОТЕКТИВНЫХ СОЕДИНЕНИЙ В РЯДУ НОВЫХ ПРОИЗВОДНЫХ ЭТИЛТИАДИАЗОЛА

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RESEARCH ARTICLE

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Цель: Поиск нейропротекторов в ряду новых производных этилтиадиазола в условиях экспериментальной черепно-мозговой травмы

Материалы и методы. Эксперимент проведен на 78 белых крысах-самцах 270±20 г линии «Wistar» 5–6-месячного возраста и 120 аутбредных половозрелых мышах массой 20±2 грамма. Исследована острая токсичность соединений. Произведен фармакологический скрининг производных этилтиадиазола с изучением поведенческого статуса и неврологических реакций. Тяжесть черепно-мозговой травмы оценивалась по шкале неврологического дефицита McGraw в модификации И.В. Ганнушкиной и шкале mNSS. Для оценки поведенческого статуса животных использовали тесты «Открытое поле» и «Rota-rod».

Результаты. Выявлено соединение-лидер – ЛХТ 12–18 в дозе 50 мг/кг. При фармакологической коррекции черепно-мозговой, данное соединение имело самый низкий процент летальности среди исследуемых соединений (8%), статистически значимо снижалась тяжесть неврологического дефицита, регистрировали самые низкие баллы и более высокий уровень моторной деятельности конечностей. Количество стоек в группе животных, получавших соединение ЛХТ 12–18, увеличивалось в 1,5 раза статистически значимо ($p<0,05$), относительно группы контроля. Исходя из результатов теста «Rota-rod», суммарное время удержания животных на стержне за 3 попытки статистически значимо отличалось в группах с применением производных ЛХТ 12–18 (в 1,5 раза дольше) в сравнение с контролем ($p<0,05$).

Заключение. Экспериментальным путем было доказано наличие нейропротективных свойств у производного этилтиадиазола ЛХТ 12–18 в дозе 50 мг/кг у крыс.

Ключевые слова: черепно-мозговая травма, производные этилтиадиазола, нейропротекция

Список сокращений: ЧМТ – черепно-мозговая травма; ЛХТ – шифр производного этилтиадиазола (4–15, 10–18, 11–18, 12–18); АТФ – аденозинтрифосфат; ДАТ – диффузная аксональная травма; тСАК – травматическое субарахноидальное кровоизлияние; ГЭБ – гематоэнцефалический барьер; ЛП – латентный период

INTRODUCTION

Home and industrial traumatism, including traumatic brain injury (TBI) is which are the main public health problem diseases in all industrialized countries and lead to persistent disability, as well as high mortality, disability and lead to high treatment costs [1].

The development of effective pharmacological correction ways of traumatic brain injury consequences is one of the main tasks of experimental research, which for highly developed countries spend billions dollars [2]. Unfortunately, many substances give excellent results only at the stage of research in the laboratory. The reason for this is the complex pathogenesis of TBI, which includes a complex of interrelated factors that affect the primary and secondary “wave” of damaging brain processes [3]. Therefore, the searching for innovative neuroprotectors is relevant in the modern science.

Traumatic brain injury can be classified into severity: mild, moderate, and severe categories. Also the special forms of contusion are distinguished: diffuse axonal injury (DAI) and traumatic subarachnoid hemorrhage. The most common is moderate brain contusion.

The overwhelming majority of patients with TBI are diagnosed with deviations of varying severity – from minor disorders to pronounced neurological symptoms. TBI in the long-term period can be a trigger mechanism for the development of diseases such as Parkinson’s and Alzheimer’s. In the first 10–12 months after an injury, the risk of an epileptic seizure is very high (12 times). Post-traumatic epilepsy is detected in more than 10% of patients with moderate pathology [5].

TBI are classified into primary and secondary. Primary brain injury is a result of a traumatic factor on the bones of the skull including structures and vessels of brain [6]. These lesions occur due to various kinds of impact, which characterizes a wide range of damage reactions.

TBI causes damage to the cells of the nervous system, the constituent structures of the vessels, the structures of white matter. This entails the onset of the second wave of damage – stress for metabolic processes, as well as disturbances in ion exchange, biochemical and molecular levels of neuronal regulation [7–9].

The metabolism in nerve cells after TBI is increased: the reserves of adenosine triphosphate (ATP) are depleted, the Ca^{2+} pump are disrupted. The increased permeability of cell membranes for Ca^{2+} leads to the release of calcium from the intracellular calcium depot. Cell depolarization and glutamate release of nerve endings leads to a violation of the membranes integrity of nerve cells and vascular endothelium [6, 10, 11]. The neurotransmitter (glutamate) provokes the activated postsynaptic glutamate receptors. The increase in the Na^{+} influx leads to a further depolarization. More Ca^{2+} start entering the cell through ion channels. The consequence of calcium overloading cells damaged by the activation of phospholipases, proteases and nucleases are leads to the loss of membrane integrity, genome expression, and destruction of the structural components in cell [12, 13].

TBI primary injuries includes local brain contusion, cerebral trunk contusion, axonal and vessel cerebral injuries. Primary injury affects of the neurons body and astrocytes, synaptic breaks, in the vessels formed blood clots and the walls of the vessels is disrupted. At the end of the pathological process of primary trauma, a decrease in the supply of adenosine triphosphate (ATP) occurs due to a violation of the permeability of cell membranes, which at the next stage leads to cytotoxic edema and cell death. [14, 15].

A penumbra zone forms along the periphery of the primary injury. All cells remain viable. Only their sensitiv-

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ity to even minor deviations in the normal operation of oxygen and nutrient delivery increases [16, 17].

Mechanical neuronal membranes destruction is a triggering factor leading to the depletion of ionic reserves of cell, free radicals and lipid peroxidation are formed. The next pathological stage is an increase Ca^{2+} content in cell, the triggering of phospholipases and calpain. All these pathological factors activate secondary injury to the membrane and cytoskeleton of neurons. Plasma movement of the axon is slowed down and leads to delayed cell death [18].

Due to TBI apoptosis of nerve cells is triggered. This process begins with an action of damaging agent on the cell genome or with the destructive action of inflammatory mediators. The influence of secondary brain injury factors, the transport of O_2 and nutrients to neurons is disrupted, as well as their destruction begins in an unnecessary volume [19, 20]. The prenumbra area is more susceptible to pathological changes due to TBI [21].

The inflammatory response is triggered out as a result of TBI primary injury. These reactions are damaging and neuroprotective. The primary pathological processes in TBI are triggered due to any mechanical damage of the cerebral tissue. Scientists attach great importance to secondary trauma in animal experiments. And that's why, pharmacological approaches to the treatment of the consequences of TBI, affecting the secondary pathology mechanisms with further apoptosis of brain cells, require in-depth study [9].

The development of secondary disorders after TBI are cause why inhibition of cerebral microcirculation, violations of oxygenation and metabolic processes in nerve cells are observed, and also occurs edema and cerebral ischemia (CI). These damages occurs on a 40% of people who had sufferred moderate TBI and 85% of severe TBI [22].

People who had suffered from an TBI, the risk of an unfavorable outcome in the event of secondary brain injury increases, since that worsens the severity of the patient's condition and restoration of cognitive functions. Therefore, timely prophylaxis and correctly chosen treatment of secondary brain injury is the main task of therapy for victims of TBI severe [9].

The inflammatory response occupies one of the most important parts, an evolutionarily process of tissue reactions. A membrane-destroying process at the cels, due both mechanical damage and autolytic processes. The end of such pathological processes can be necrosis and apoptosis or regeneration and repair [23]. The reconstructions of cells involves all factors of inflammatory response. These factors including: edema, inhibition of blood circulation and protein, carbohydrate, fat metabolism. The fact that sanogenic pathological reactions such as edema and hyperemia, in the case of generalization, can have a pathogenic or even thanatogenic character.

In case of primary brain injury, activation and release of large volumes of cytokines begins throughout the human body. These cytokines can be inflammatory and anti-inflammatory. Also, an activation of resident macrophages from astrocytes with microglia in brain, the movement of neutrophils to the injury and to the violations of BBB permeability. The cytokines consists of: growth factors, interleukins, neuropoietins, chemokines, interferons, tumor necrosis factors, neurotropins. All these components are involved in the inflammatory response [24].

Despite the rapid development of experimental pharmacology [4, 25–28], the improvement of methods of directed synthesis, allowing the creation of highly selective drugs, remains highly relevant for new compounds. Ethylthiadiazole derivatives may be potential candidates with neuroprotective properties. Among them, a large number of compounds with anti-inflammatory, antimicrobial, anticonvulsant, hypotensive, antioxidant, and antitumor effects have been identified [29–34].

THE AIM of the study: to study the neuroprotective properties of new ethylthiadiazole derivatives under the conditions of experimental traumatic brain injury (TBI).

MATERIALS AND METHODS

Test samples

LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 were synthesized at the Russian Research Center for Safety of Bioactive Substances (Staraya Kupavna, Russia).

Animals

The experiment was conducted on 78 white male Wistar rats aged 5–6 months, body weight 270±20 gramms and in 120 outbred white mice with body weight 20±2 gramms. The animals were buyed at the Federal State Institution of Science Scientific Center of Biomedical Technology of the Federal Medical-Biological Agency of Russia. Conditions of detention: under standard conditions in accordance with Sanitary and epidemiological requirements for the device, equipment and maintenance of experimental biological clinics (vivariums) No2.2.1.3218-14 and Federal Standard of Russia No.33044-2014.

Study design

The acute toxicity of LKHT 4–15, LKHT 10–18, LKHT 11–18 and LKHT 12–18 was studied in male mice. The next dose range was studied: 500 mg/kg, 1000 mg/kg, 2500 mg/kg, 5000 mg/kg. The choose of doses was carried out by the experiment. The injection of the samples was made fractionally. Each mouse were observed keep watching for the first 60 minutes after injection of the test samples. Then the observation was performed once per day.

The manipulations on rats were carried out under general anesthesia by intraperitoneal injection of chloral hydrate (300 mg/kg). In this work, the technology of modeling TBI on rats was reproduced by the method of free-fall of block with weight 155 grams from height at 0.6 meters [33]. The setup consist of a stand with a 1.1 meters long hollow tube clamped in a stand in a vertical position. A firing pin with stopper is locate at lower edge of the pipe. The striking surface area of firing pin was 0.5 cm^2. The movement of the firing pin was limited in any conditions to no more than 5 mm. With the help of a return spring, upon impact, the firing pin returns to starting point. Restriction in the movement of the firing pin allows to escape depressed fractures of the cranial vault. The block was placed in the pipe cavity at a prearranged height. The displacement of the firing pin at the point of impact was excluded if using this function in experiment. Before modeling the pathology, it was checked the position of the pipe (installed vertically) and the table (horizontally). The localization of the impact was carried out according to the anatomy of the rat brain. The impact was simulated in the field zone Fr1, Fr3, FL, HL, Par1, Pag2. The impact site was located in the fronto-parietal-temporal region of the left hemisphere of the brain. The rat's head was not rigidly fixed. This model made it possible to reproduce the TBI model as close as possible to that in humans [33].

The pharmacological screening was carried out using the severity of neurological deficits by the McGraw point scale modified by I.V. Gannushkina [35] and the mNSS scale [36].

This McGraw scale as modified by I.V. Gannushkina (Table 1) is presented with a list of neurological disorders. The analyzed indicators were summarized. Depending on the total amount, the neurological deficit can be designated in different ways: the amount of points 0.5-2.0 corresponds to a mild degree of deficiency; 2.5–5.0 – moderate severity; 5.5-10 severe neurological deficits. The evaluation is carried out on the 1st, 3rd and 7th days of the experiment.

To assessment the neurological deficit, a modified test for neurological deficit was carried out 48 h after modeling the TBI. The mNSS scale (modified neurological severity score) is a special system for interpreting neurological deficits in the presence of a brain injury. It is used to assess motor, sensory, balance, and reflex behavior of animals [36].

According to this neurological scale, rodents were suspended by the tail (to determine the presence of paresis and paralysis), motor activity in the home cage (to register gait disturbances and stereotypical movements) and the peculiarities of movement on a horizontal bar (to assess the coordination of movements) were assessed, the safety of the main reflexes (startle reflex, reflex of the external auditory canal, corneal reflex). The results of the study to determine the neurological deficit were formulated based on the sum of points scored in each test. A higher score indicates a more severe injury. The total number of points in the range from 1–6 indicates the presence of a mild TBI, from 7 to 12 – moderate, and a total of 13–18 indicates the presence of severe TBI.

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To the behavioral assessment of animals, the Open Field [37] and Rota-rod [38] tests were used.

Open Field Test

This behavioral test is used to register the characteristics of behavioral reactions in pharmacology and psychogenetics. The main task of this testing is to study the motor component of the orienting reaction and emotional reactivity of rodents. The setup for the Open Field Test has a large number of modifications, and the parameters of determination in this test depend from objectives of study. In rodents without pathologies, exploratory behavior prevails over fear, therefore, if the rodent is placed in the arena, with normal levels of horizontal and vertical activity, exploratory behavior dominates over fear (grooming and bowel movements). The analysis of the behavioral status with the exploratory activity assessment of the rats was investigated in an "open field" installation (Open Field LE800S, PanLab Harvard Apparatus, Spain) for 10 minutes. The room was illuminated with a 100W lamp, which hung at a height of 1.5 m from the bottom of the arena. The rodents were placed an "open field" around the periphery of the arena. The arena was wiped with a damp cloth after each animal. For analyzing the data obtained, the Smart v.3.0.03 program was used. (Panlab Harvard Apparatus, Spain). The results of the data obtained were summarized.

For statistical calculations of the "Open Field" test, the following indicators were taken: horizontal activity in the center, horizontal activity on the periphery, stances, mink reflex, number of defecation acts, number of urination acts, grooming.

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Rota-rod Test

The Rota-rod test is used characterizing motor coordination of movements [13]. In this experiment, a constant rod rotation speed of 20 rpm was used. The latency period (LP) of first fall (the time of first fall of rat from the rotating rod) and the total holding time on the rotating rod for 3 attempts were recorded [33, 39–42].

Based on the objective, all animals were divided into the next groups (n=13):

1. Intact group (animals with oral administration of NaCl in equivalent doses)
2. Simulation of experimental TBI (control)
3. Simulation of experimental TBI + LHT 4–15
4. Simulation of experimental TBI + LHT 10–18
5. Simulation of experimental TBI + LHT 11–18
6. Simulation of experimental TBI + LHT 12–18.

All substances were injected at dose of 50 mg/kg (dose selection was carried out by the experiment), 30 minutes before the modeling of the pathology. Samples were dissolved with sodium chloride and injected intragastrically.

A day later, the indicator assessments of neurological deficit and behavioral status was researched [15].

Statistical processing of results

Descriptive statistics were used for all data. The data obtained were checked for normality of distribution. The type ща distribution was determined by the Shapiro-Wilk test. In the case of a normal distribution, the mean value (M) and the standard error of the mean (m) were calculated. Inter-group differences were analyzed using parametric (Student's *t*-test) or non-parametric (Mann-Whitney test) methods, depending on the type of distribution. Statistical analysis was performed using IBM SPSS Statistics 23 (USA) and Microsoft Office Excel 2010.

RESULTS AND DISCUSSION

During the experiment, it was found that injection of LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 to mice at the dose range of 500-5000 mg/kg did not lead to changes compared with the usual behavior of rodents. When LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 were injected at dose of 10000 mg/kg, a decrease in behavioral activity was observed and, visually, a slight increase of respiratory rate in mice was recorded. Animals were localized on the periphery of the cage. There was no change in the skin and hair, mucous membranes of mice. The amount and quality of urinations and defecations were unchanged.

Based on the data obtained during the study of acute toxicity of LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18, it was not possible to determine LD_{50}, since no deaths of mice were recorded. Maximum injected dose 10000 mg/kg was selected as LD_{50} for further experiment, according to the protocol and design of the study.

The investigated derivatives of ethylthiadiazole were injected to rats at a dose of 50 mg/kg (1/200 of maximum injected dose).

The effect of ethylthiadiazole derivatives on indicators of neurological deficit in experimental animals with simulated TBI

For all animals of the experimental groups, the characteristic symptoms were: lethargy, tremor, weakness of the limbs, paresis. The cognitive dysfunction rats was recorded, pathological work of the forelimbs was observed: the animal pulled the forelimbs along with it, the fingers were clenched to the palm.

At the first days of experiment, the mortality rate was equal to 0% in all groups. On the 3rd day, the highest percentage of mortality – 23%, was in the groups with TBI without correction, with correction of LKHT 10-18 and LKHT 11–18 at the dose of 50 mg/kg. The lowest percentage (8%) of fatality on the 3rd day was registered in the group of animals injected by LKHT 12–18.

The highest mortality rate of animals in experimental groups was observed for 3 to 7 days. TBI caused the death of a high percentage of control group animals (46%). Injection of LKHT 11–18 at the dose of 50 mg/kg did not lead to a significant reduction in the number of deaths (38%). Average mortality rates were recorded in groups with LKHT 10–18 (31%) and LKHT 4–15 (23%). The lowest mortality rate was in group injected by the LKHT 12–18 (8%).

The effectiveness of LKHT 4–15, LKHT 10–18, LKHT 11–18, and LKHT 12–18 at the dose of 50 mg/kg on the neurological deficit of animals after TBI simulation was evaluated using the McGraw stroke score scale modified by I.V. Gannushkina and the psychometric neurological deficit score scale mNSS. The intact group didn't have neurological deficit.

The group without pharmacological treatment was taken as a control.

In this group, a neurological deficit of moderate severity was observed on 1st day – 4.04 points, with a tendency to worsen the severity by day 7th to 6.08 points.

One day after modeling the pathology, a severe degree of neurological deficit was recorded in the control group (4.04 points). In the group LKHT 12–18, the neurological deficit was mild (2.96 points). In groups with correction, LKHT 4–15, LKHT 10–18 and LKHT 11–18 occupied intermediate values (3.19, 3.65 and 3.88 points).

On the 3rd day, a neurological status decrease was observed in all groups, with the exception of the group with correction LKHT 12–18 (2.73 points).

On the 7th day, a neurological deficit decrease was observed in the groups with LKHT correction 12–18 (2.46 points) and using of Citicoline at a dose of 500 mg/kg (2.97 points). In the group LHT 4–15, the severity did not change (4.27 points). In the control groups LHT 10–18 and LHT 11–18, the severity changed to severe (6.08, 5.54 and 5.73 points).

Table 1 – Scale for neurological deficit according to McGraw modified by I.V. Gannushkina (1996)

Symptoms	Point
Lethargy, slowness of movement	0.5
Tremor	1.0
Unilateral half-tosis	1.0
Bilateral half-tosis	1.5
Unilateral ptosis	1.5
Bilateral ptosis	1.5
Manege movements	2.0
Paresis of 1–4 limbs	2.0–5.0
Paralysis of 1–4 limbs	3.0–6.0
Coma	7.0
Fatal outcome	10.0

Table 2 – Modified mNSS neurological symptom severity scale

Test	Point	Manifestations	Point
Hanging by the tail	0–3	Flexion of the forelimb	1
		Flexion of the forelimb	1
		Head displacement >10° from vertical axis for 30 sec	1
Physical activity	0–3	Without features	0
		Impossibility to move in a straight line	1
		Arena movements	2
		Falling to one side	3
Sensory tests	0–2	Front limb placement test	1
		Resistance to passive flexion of the limb in the ankle joint	1
Walking the crossbar	0–6	Steady posture	0
		Pinching one side of the bar	1
		Grasping the bar with sliding one of the limbs	2
		Grasping the bar with slipping of two limbs or rotating on the bar (> 60 sec)	3
		Unsuccessful attempt to stay on the bar, fall (>40 sec)	4
		Unsuccessful attempt to hold onto the bar, fall (> 20 sec)	5
		Falling without trying to hang or hold onto a beam (<10 sec)	6
Loss of reflexes, specific movements	0–4	Reflex of the external auditory canal	1
		Corneal reflex	1
		Startle reflex	1
		Convulsions, myoclonus, muscular dystonia	1

Table 3 – The effect of ethylthiadiazole derivatives on the dynamics of neurological disorders severity in accordance with McGraw score scale modified by I.V. Gannushkina (1996) (by the average score) (n=13)

Groups	Days		
	1	3	7
Intact animals	0	0	0
TBI	4.04	5.35	6.08
LKHT 4–15 (50 mg/kg)	3.19	4.00	4.27
LKHT 10–18 (50 mg/kg)	3.65	4.69	5.54
LKHT 11–18 (50 mg/kg)	3.88	4.85	5.73
LKHT 12–18 (50 mg/kg)	2.96	2.73*	2.46*
Citicoline(500 mg/kg)	3.97	4.33	2.97*

Note: * – $p<0.05$ in comparison with the control group of rats

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Table 4 – The effect of ethylthiadiazole derivatives on the dynamics of neurological disorders severity in accordance to the mNSS neurological assessment deficit scale (based on the average score) (n=13)

Groups	Period of time
	2nd day
Intact animals	0
TBI	10.69
LKHT 4–15 (50 mg/kg)	8.38*
LKHT 10–18 (50 mg/kg)	8.92
LKHT 11–18 (50 mg/kg)	9.83
LKHT 12–18 (50 mg/kg)	7.85*
Citicoline(500mg/kg)	8.00

Note: * – p<0.05 in comparison with the control group of rats

Table 5 – Results of the Open Field Test with intragastric injecttion of ethylthiadiazole derivatives (M±m; n=13)

Test indicatirs / Groups	Horizontal activity in the center, (m)	Horizontal activity in the outer zone, (m)	Rearing behavior	Hole Exploratory behavior	Grooming	Defecation	Urination
Intact animals	0.66±0.23	71.72±3.30	10.77±0.60	4.69±0.36	14.15±0.96	2.00±0.30	0,92±0,21
TBI	0.58±0.13	38.34±7.98	3.55±0.53	2.90±0.31	15.72±0.93	0.73±0.24	0.55±0.20
LKHT 4–15 (50 mg/kg)	1.25±0.20*	53.57±5.90	4.42±0.81	2.83±0.47	13.67±0.85	1,08±0,26	0.42±0.20
LKHT 10–18 (50 mg/kg)	0.77±0.09	55.92±6.29	3.90±0.45	3.09±0.65	14.18±0.86	0,91±0,25	0.55±0.21
LKHT 11–18 (50 mg/kg)	0.69±0.07	42.09±2.44	3.82±0.54	2.63±0.53	15.09±0.62	0,82±0,26	0.72±0.25
LKHT 12–18 (50 mg/kg)	1.5±0.11*	59.47±3.41*	5.92±0.38*	3.41±0.37	14.50±0.72	1,00±0,25	0.83±0.21
Citicoline (500 mg/kg)	1.44±0.22*	49.8±3.00*	4.60±0.53	3.40±0.35	12.78±0.60*	1,00±0,28	0.74±0.21

Note: * – p<0.05 in comparison with the control group of rats

Table 6 – Results of the Rota-rod Test with intragastric administration of the ethylthiadiazole derivatives (M±m; n=13)

Group	The latent period of the first fall	The total retention time of animals for 3 attempts
	72 hours	
Intact group	83.31±2.86	158.69±2.13
TBI	7.64±0.61	68.90±4.54
LKHT4–15	41.25±3.20*	95.58±1.09*
LKHT10–18	30.67±2.46*	81.18±1.33
LKHT11–18	24.18±1.98*	81.00±3.78
LKHT 12–18	49.83±3.39*	105.08±1.89
Citicoline(500 mg/kg)	35.73±3.65*	89.14±2.50*

Note: * – p<0.05 in comparison with the control group of rats

Injection of LHT 4–15 and LHT 12–18 with simulated TBI resulted in a marked decrease in the severity of neurological deficit in comparison with the control. Statistically significant (p<0.05) improvement in the severity of neurological deficit was registered in the group of animals injected by the LHT 12–18 at the dose of 50 mg/kg in comparison with the control group, the data obtained presented in table 3.

On the 2nd day, the rats injected by the LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 at the dose of 50 mg/kg had more mildsymptoms of neurological deficit in accordance with mNSS scale in comparison with the control group of animals. But statistically significant differences (p<0.05) was observed only in groups LKHT 4–15and LKHT 12–18 at the dose of 50 mg/kg, data obtained presented in table 4. Rats of these groups had more pronounced motor skills compared with control group.

Besides the results of neurological deficit on two scales of pronounced neuroprotective activity in LKHT 4–15 and LKHT 12–18 at the dose of 50 mg/kg was revealed. In rodents of these groups, the lowest scores of neurological deficits and a higher level of motor activity of the limbs were recorded. The statistically significant differences in the two scales and the level of lethality LKHT 12–18 was identified as a leader.

The effect of new ethylthiadiazole derivatives on the indicators of the behavioral status in simulated TBI

The Open Field Test, the motor and exploratory ac-

tivity of intact animals was evaluated, as well as animals with simulated TBI without pharmacological treatment and administered with ethylthiadiazole derivatives. The data obtained presented in table 3.

Among the Open Field testing 72 hours after injury simulation showed that pharmacological treatment, motor and exploratory activity in comparison with the intact group was lower by 2 times and 3 times, respectively; movement in the field was chaotic with extremely rare peeks into holes and rearing behaviors, the rats did not investigated the entire area of the field.

Motor activity significantly decreased in all groups relative to intact animals. The LKHT 12–18 at the dose of 50 mg/kgcould significantly hinder the reduction of motor activity in the animals on the background of a traumatic brain injury, $p<0.05$ in comparison with the indicators of the control group from all groups.

The evaluation of exploratory activity was also decreased in all groups in comparison with intact animals. In the control group, the largest decrease in this indicator was still observed; the data obtained presented in table 5. Hole exploratory behavior in the groups injected by the LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 did not decrease significantly, relative to the control group of animals. The number of rearing behaviors in the injected group by the LKHT 12–18 at the dose of 50 mg/kg increased by 1.5 times, statistically significant ($p<0.05$) relative to the control group. The number of defecations and urinations decreased not statistically significant. Grooming was statistically significantly different from the control in all groups ($p<0.05$).

The Rota-rod Test, the control group recorded a decrease in the retention time of animals on a rotating rod for 1 attempt (latent period) and 3 attempts, in comparison with the intact group, the data obtained presented in table 6. TBI simulation of the control group caused significant violations of strength and coordination. Regression of the total retention time relative to the intact group was determined. The injecttion of all ethylthiadiazole derivatives LKHT 4–15, LKHT 10–18, LKHT 11–18, LKHT 12–18 at the dose of 50 mg/kg significantly increased the retention time for 1 attempt on the rod ($p<0.05$) in comparison with the control. The highest indicators in this test for 1 attempt of retention were registered in groups of LKHT 4–15 – 5.5 times, LKHT 12–18 – 7 times higher than in the control. The lowest rates were observed in the group with TBI and LKHT 11–18 – only 4 times.

The total retention time of animals on the rod for 3 attempts statistically significant differences in groups LKHT derivatives 4–15 (1.4 times longer), LKHT 11–18 (1.25 times longer), LKHT 12–18 (1.5 times longer) at the dose of 50 mg/kg compared with the control ($p<0.05$).

Based on the results of the Rota-rod Test, the most promising compound for pharmacological treatment of the consequences of traumatic brain injury is LKHT 12–18 at the dose of 50 mg/kg.

DISCUSSION

For identification a promising neuroprotective compound among new ethylthiadiazole derivatives, the most significant and reliable indicators were found in the compound LKHT 12–18 at the dose of 50 mg/kg: the lowest percentage of mortality among groups with pharmacological treatment of pathology (8%); LKHT 12–18 administration to rats with simulated TBI led to a significant decrease in the severity of neurological deficit in comparison with the control, the decrease of the severity of neurological deficit in the group of animals administered with LKHT 12–18 at the dose of 50 mg/kg was statistically significant ($p<0.05$) in comparison with the control group; the evaluation of neurological deficit indicators using mNSS scale showed a pronounced neuroprotective activity of LKHT 12–18 at the dose of 50 mg/kg. In rodents of this group, the lowest scores of neurological deficits and a higher level of motor activity of the limbswere recorded. The number of rearing behaviors in the group of animals injected by LKHT 12–18 at the dose of 50 mg/kg increased by 1.5 times, statistically significant ($p<0.05$) relative to the control group. Based on the results of the Rota-rod Test, the total retention time of animals on the rod for 3 attempts significantly differed in the groups LKHT 4–15 (1.4 times longer), LHT 11–18 (1.25 times longer), LHT 12–18 (1.5 times longer) at the dose of 50 mg/kg compared with the control ($p<0.05$).

Based on the results obtained in this study, it is planned to study in more detail the LKHT 12–18 at the dose of 50 mg/kg using a complex of biochemical and morphometric research methods that will suggest a potential mechanism of action of this leader compound.

CONCLUSION

LHT 12–18 – an ethylthiadiazole derivative at a dose of 50/mg has the most pronounced neuroprotective effect in an experimental model of traumatic brain injury. Based on the results obtained in this study, a more detailed study of LHT 12–18 is planned using a complex of biochemical and morphometric research methods, which will suggest a potential mechanism of action of this leader compound.

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AUTHORS' CONTRIBUTION

All authors equally contributed to the research work.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Terry DP, Brassil M, Iverson GL, Panenka WJ, Silverberg ND. Effect of depression on cognition after mild traumatic brain injury in adults. Clin Neuropsychol. 2019;33(1):124–136. DOI: 10.1080/13854046.2018.1459853
2. Courtney A, Courtney M. The Complexity of Biomechanics Causing Primary Blast-Induced Traumatic Brain Injury: A Review of Potential Mechanisms. Front Neurol. 2015;6:221. DOI: 10.3389/fneur.2015.00221
3. Agoston DV, Sköld MK. Editorial: When Physics Meets Biology; Biomechanics and Biology of Traumatic Brain Injury. Front Neurol. 2016 Jun 15;7:91. DOI: 10.3389/fneur.2016.00091
4. Beskhmelnitsyna EA, Pokrovskii MV, Kulikov AL, Peresypkina AA, Varavin EI. Study of Anti-inflammatory Activity of a New Non-opioid Analgesic on the Basis of a Selective Inhibitor of TRPA1 Ion Channels. Antiinflamm Antiallergy Agents Med Chem. 2019;18(2):110–125. DOI: 10.2174/1871523018666190208123700
5. Vasserman LI, Sergeev VA. Otnoshenie lichnosti k bolezni pri cerebral'noj travme i travmaticheskoj epilepsii. Aktual'nye problemy somatopsihiatriii i psihosomatiki. Moscow. 1990:47–49. Russian
6. Gomazkov OA. Neurochemistry of ischemic and age-related brain pathologies [Text]. Moscow, 2003: 200p.
7. Gusev EI, Konovalov AN, Skvortsova VI Neurology and neurosurgery [Text]. Moscow. 2004:420p. Russian
8. Danbolt NC. Glutamate uptake. Prog Neurobiol. 2001;65(1):1–105. DOI: 10.1016/s0301-0082(00)00067-8
9. Karakulova Yu.V., Selyanina N.V., Eroshina O.A. Quality of life of patients in the acute period of traumatic brain injury under the influence of neurotrophic therapy. Bulletin of Siberian Medicine. 2011; 10(2):122–126. Russian
10. Castillo J, Dávalos A, Alvarez-Sabín J, Pumar JM, Leira R, Silva Y, Montaner J, Kase CS. Molecular signatures of brain injury after intracerebral hemorrhage. Neurology. 2002;58(4):624–9. DOI: 10.1212/wnl.58.4.624
11. Semenza GL, Agani F, Feldser D, Iyer N, Kotch L, Laughner E, Yu A. Hypoxia, HIF-1, and the pathophysiology of common human diseases. Adv Exp Med Biol. 2000;475:123–30. DOI: 10.1007/0-306-46825-5_12.
12. Sirovsky EB, Amcheslavsky VG, Kulikovsky VP, EB Sirovsky. Mechanisms of development of cerebral edema in neurosurgical pathology [Text]. Vesti AMS USSR. 1991, No. 7. P. 7–13.
13. McEvoy LK, Fennema-Notestine C, Eyler LT, Franz CE, Hagler DJ Jr, Lyons MJ, Panizzon MS, Rinker DA, Dale AM, Kremen WS. Hypertension-related alterations in white matter microstructure detectable in middle age. Hypertension. 2015;66(2):317–23. DOI: 10.1161/HYPERTENSIONAHA.115.05336
14. Fiskum G. Mitochondrial participation in ischemic and traumatic neural cell death. J Neurotrauma. 2000;17(10):843–55. DOI: 10.1089/neu.2000.17.843
15. Richard KE. (1991) Traumatic Brain Swelling and Brain Edema. In: Frowein R.A. (eds) Celebral Contusions, Lacerations and Hematomas. Advances in Neurotraumatology (Published under the Auspices of the Neurotraumatology Committee of the World Federation of Neurosurgical Societies). Springer, Vienna. 1991;3:101–139. DOI:10.1007/978-3-7091-6922-3_5
16. Orth M, Schapira AH. Mitochondria and degenerative disorders. Am J Med Genet. 2001 Spring;106(1):27–36. DOI: 10.1002/ajmg.1425.
17. Sims NR, Anderson MF. Mitochondrial contributions to tissue damage in stroke. Neurochem. Int. 2002, Vol. 40, No.6. P. 511–526.
18. Gusev EI., Skvortsova VI. Neuroprotective therapy for ischemic stroke. I Primary neuroprotection [Text]. Journal of Neurology and Psychiatry. S.S. Korsakova (Stroke). 2002; 5:3–16. Russian
19. Zavalishin IA, Zakharova MN. The death of a neuron is a cardinal problem of neurology and psychiatry [Text]. Bulletin of the RAMS. 1999; 1:28–33. Russian
20. Zozulya, YuA., Baraboy VA, Sutkova DA Free radical oxidation and antioxidant protection in brain pathology [Text]. Zozulya. 2000:344 p. Russian
21. Gusev EI, Krylov VV. Cerebral ischemia [Text]. Emergency neurosurgery. Moscow. 2001:327.
22. Likhterman LB, Potapov AA, Kravchuk AD. Modern approaches to the diagnosis and treatment of traumatic brain injury and its consequences [Text]. Neurosurgery issues. 1996;(1).3537.
23. Wallach D, Kang T, Dillon C, Green D. Programmed necrosis in inflammation: Towards identification of the effector molecules. Science. 2016; 352:2154.
24. Weissman C. The metabolic response to stress: an overview and update. Anesthesiology. 1990;73(2):308–27. DOI: 10.1097/00000542-199008000-00020
25. Pobeda AS, Miller ES, Soldatov VO, Lazareva GA, Reznikov KM, Sernov LN, Yakovleva EG, Reznichenko LV, Faitelson AV. The research of abnormal toxicity and local irritant effect in the draize test of the drug furacilin, concentrate for the preparation of a solution for local and external use. Journal of International Pharmaceutical Research.2019;46(4):262–266. Russian
26. Peresypkina AA, Pokrovskii MV, Gubareva VO, Dolzhikov AA. Effect of carbamylateddarbepoetin on the model of ischemic neuropathy of the optic nerve in rats. Eksperimental'nayai Klinicheskaya Farmakologiya. 2018, 81(7):8–13. Russian
27. Peresypkina AA, Pokrovskii MV, Dolzhikov AA, Levkova EA, Pobeda AS. Correction of experimental ischemic neuropathy of the optic nerve by imidazoline receptor agonist type I and II. Eksperimental'nayai Klinicheskaya Farmakologiya. 2018;81(4):12–17.
28. Peresypkina A, Pazhinsky A, Danilenko L, Lugovskoy S, Pokrovskii M, Beskhmelnitsyna E, Solovev N, Pobeda A, Korokin M, Levkova E, Gubareva V, Korokina L, Martynova O, Soldatov V, Pokrovskii V. Retinoprotective Effect of 2-Ethyl-3-hydroxy-6-methylpyridine Nicotinate. Biology (Basel). 2020;9(3):45. DOI: 10.3390/biology9030045
29. Schenone S, Bruno O, Ranise A, Bondavalli F, Filippelli W, Falcone G, Giordano L, Vitelli MR. 3-Arylsulphonyl-5-arylamino-1,3,4-thiadiazol-2(3H)ones as anti-inflammatory and analgesic agents. Bioorg Med Chem. 2001;9(8):2149–53. DOI: 10.1016/s0968-0896(01)00121-3
30. Abdel-Wahab BF, Abdel-Aziz HA, Ahmed EM. Synthesis and antimicrobial evaluation of some 1,3-thiazole, 1,3,4-thiadiazole, 1,2,4-triazole, and 1,2,4-triazolo[3,4-b][1,3,4]-thiadiazine derivatives including a 5-(benzofuran-2-yl)-1-phenylpyrazole moiety. Monatsh Chem. 2009;140:601–605. DOI:10.1007/s00706-008-0099-x
31. Almajan GL, Innocenti A, Puccetti L, Manole G, Barbuceanu S, Saramet I, Scozzafava A, Supuran CT. Carbonic anhydrase inhibitors. Inhibition of the cytosolic and tu-

mor-associated carbonic anhydrase isozymes I, II, and IX with a series of 1,3,4-thiadiazole- and 1,2,4-triazole-thiols. Bioorg Med Chem Lett. 2005 May 2;15(9):2347–52. DOI: 10.1016/j.bmcl.2005.02.088

32. Amir M, Kumar H, Javed S. Condensed bridgehead nitrogen heterocyclic system: synthesis and pharmacological activities of 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives of ibuprofen and biphenyl-4-yloxy acetic acid. Eur. J. Med. Chem. 2008, 43:2056–2066.
33. Martynova OV, Antsiferov OV, Martynov MA, et al.The study of neurodynamic disturbances in rats with cranial injury. Research Results in Biomedicine. 2019;5(3):50–63. DOI:10.18413/2658-6533-2019-5-3-0-6. Russian
34. Liu W, Chen Y, Meng J, Wu M, Bi F, Chang C, Li H, Zhang L. Ablation of caspase-1 protects against TBI-induced pyroptosis in vitro and in vivo. J Neuroinflammation. 2018;15(1):48. DOI: 10.1186/s12974-018-1083-y
35. McGraw CP. Cerebral infarction in the Mongolian gerbil exacerbated by phenoxybenzamine treatment. Stroke. 1976;7(5): 485–488.
36. Khudyakova NA, Bazhenova TB. Behavioural activity of linear and nonlinear mice of different color variations in the test «open field». Bulletin of Udmurt University.Series Biology. Earth Sciences [Vestnik Udmurdskogo Universiteta. Seriya Biologiya. Nauki o zemle]. 2012,2:89–93 Russian
37. Bohlen M., 2009. Calibration of rotational acceleration for the rotarod test of rodent motor coordination. Journal Neurosci. Methods, 178 (1):10–4.
38. Mironov AN et al. Guidelines for pre-clinical trials of medicines. Moscow: Grif and K. 2012; (1):944. Russian
39. Fortier CB, Amick MM, Kenna A, Milberg WP, McGlinchey RE. Correspondence of the Boston Assessment of Traumatic Brain Injury-Lifetime (BAT-L) clinical interview and the VA TBI screen. J Head Trauma Rehabil. 2015;30(1):E1–7. DOI: 10.1097/HTR.0000000000000008
40. Kim G, Gan'shina T, Kurza E, KurdyumovI, Maslennikov D, Mirzoian R. New cerebrovascular agent with hypotensive activity. Research Results in Pharmacology. 2019,5(2):71–77. DOI: 10.3897/rrpharmacology.5.35392
41. Avdeeva NV, Sidorova SA, Gudyrev OS, Osipova OA, Golubev IV. Mechanism of neuroprotective effect of mGluR4 agonists. Research Results in Pharmacology. 2019, 5(2): 43–47. DOI:10.3897/rrpharmacology.5.36565
42. Nesterova NI, Shcheblykina OV, Kolesnichenko PD, Nesterov AV, Shcheblykin DV, Yakovlev D. Neuroprotective effects of taurine and 3-hydroxypyridine derivatives in the intracerebral hemorrhage model in rats. Research Results in Pharmacology. 2019,5(3):87–94. DOI: 10.3897/rrpharmacology.5.36988
43. Berezovskaya IV. Forecasting of medicinal products safety in preclinical toxicological studies. Toxicological review. 2010, 5 (104):17–22. Russian

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