December 30, 2019

PharmacologyOnLine

Archives • 2019 • vol.3 • 36-45

PYRIMIDINE-4H-1OH DERIVATIVES RESTORE MITOCHONDRIAL FUNCTION IN EXPERIMENTAL CHRONIC TRAUMATIC ENCEPHALOPATHY

Pozdnyakov D.I.[1], Voronkov A.V.[1], Miroshnichenko K.A.[1], Adzhiahmetova S.L.[1], Chervonnaya N.M.[1], Rukovitcina V.M.[1]

[1]-Pyatigorsk medical and pharmaceutical Institute-branch of Volgograd state medical University of the Ministry of health of the Russian Federation (Pyatigorsk, Russia (357532, Pyatigorsk, av. Kalinina 11).

*pozdniackow.dmitry@yandex.ru

Abstract

Chronic traumatic encephalopathy is a degenerative disease of the central nervous system, the treatment of which is one of the significant problems of modern medicine. Chronic traumatic encephalopathy was modelled by the method of repeated exposure of a free-fall load to the rat's skull. The test-objects were administered for 7 days at a dose of 100 mg / kg. The change in mitochondrial function was assessed by the respiration method while determining the ATP-generating activity, the maximum level of respiration, respiratory capacity, glycolysis intensity, glycolytic capacity, glycolytic reserve. The concentration of lactic acid in serum and β-amyloid in brain tissue was also evaluated. The study found that the use of pyrimidine derivatives contributed to an increase in ATP-generating activity, the maximum level of respiration, respiratory capacity, glycolytic capacity and glycolytic reserve, as well as reducing the intensity of glycolysis, the concentration of lactic acid and β-amyloid. The content of β-amyloid with the use of DK-1, DK-2, DK-3, DK-4, DK-5 compounds decreased by 2.4 times ($p < 0.05$); 3.1 times ($p < 0.05$); 4.5 times ($p < 0.05$); 2.1 times ($p < 0.05$) and 2.5 times ($p < 0.05$), respectively. On the basis of the obtained data, it is possible to assume the promise of using compounds - pyrimidine-4H-1OH as a means of treating chronic traumatic encephalopathy, which have a metabolic profile of action and normalize mitochondrial function.

Keywords: Chronic traumatic encephalopathy, mitochondrial dysfunction, pyrimidine derivatives.

Introduction

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease that develops with repeated exposure of the brain to a traumatic factor, i.e. periodic episodes of mild or moderately severe traumatic brain injury [1]. The clinical manifestations of CTE are diverse and include: disorders of perception and behavior (delusions, hallucinations, aggressiveness), depression, suicidal tendencies, cognitive (dementia) and motor functions (parkinsonism, dysarthria) [2]. Epidemiological studies show that CTE is most often seen in contact sports athletes: American football, rugby, boxing, fighters of mixed martial arts, who have a significantly higher risk of brain concussion [3]. In addition to highly qualified athletes, CTE as a specific type of post-traumatic disorder is found in the military who have gone through the «hot» stage of armed conflict [4]. At the same time, it is significant that among patients with CTE, about 20% are people with a terminal degree of disability, usually suffering from neurodegenerative diseases and requiring specialized care, which entails an increase in the direct and indirect costs of the health-care system [5]. In the pathogenesis of CTE, mitochondrial dysfunction associated with energy deficiency is of particular importance. It has been established that mitochondrial damage arising from the effect of a traumatic factor on the brain contributes to the activation of irreversible neurodestructive processes, which are usually associated with insufficient ATP for the optimal functioning of cells, the intensification of the proapoptotic signal and oxidative stress [6]. In addition, the intracellular deficiency of high-energy compounds formed under mitochondrial dysfunction adversely affects the functional activity of other subcellular structures - the endoplasmic reticulum, and the Golgi apparatus [7]. Under these conditions, dysfunction of the Golgi apparatus leads to hyperproduction of amyloid precursor protein (APP), which in turn contributes to the activation of β-secretase and γ-secretase, resulting in the accumulation of β-amyloid in the cell - a substance that has a negative effect on the functional activity of neurons [8]. In addition to the above reactions, the abnormal mitochondria function leads to the accumulation of non-oxidation products - lactic acid, the increase in concentration of which leads to the development of acidosis, increased inflammatory reactions with an increase of cytokine synthesis (TNF-α), which in certain conditions can «close the vicious circle» of brain tissue damage by activating an external apoptotic pathway, which worsens the prognosis of the disease [9]. Thus, it can be assumed that the normalization of mitochondrial function in CTE conditions will probably contribute to the preservation of neuronal integrity and a reduction in the clinical complications of this disease.

Materials And Methods

Experimental animals.

The work was performed on 70 male Wistar rats weighing 240-260 grams. All manipulations with rats were in accordance with the requirements of the European Convention for the protection of vertebrates used for experimental and other scientific purposes (Strasbourg, 22 June 1998). The concept of the study was reviewed and approved by the local ethical committee of the Pyatigorsk Medical and Pharmaceutical Institute (Protocol No. 18 of 03/13/2019).

Study design.

During the study, the following groups of animals were formed: a group of intact rats (n = 10); negative control animals group (NC); groups of rats treated with the test-compounds (n = 10 each of 5 experimental groups). The test-objects were new pyrimidine-4H-1OH derivatives and were synthesized at the Department of Organic Chemistry of the Pyatigorsk Medical and Pharmaceutical Institute. The studied compounds were: 4- (2-methyl-6-ethyl-4-oxo-5-phenyl-4H-pyrimidin-1-yl) -N-thiazol-2-yl-benzosulfamide (code: DK-1); 4- (6-methyl-2-ethyl-4-oxo-5-phenyl-4H-pyrimidin-1-yl) -N-thiazol-2-yl-benzosulfamide (code number: DK-2); 4- (6-methyl-2-ethyl-4-oxo-5-phenyl-4H-pyrimidin-1-yl) benzosulfamide (code number: DK-3); 1- [4- (4-amino-benzenesulfonyl) -phenyl] -2-methyl-6-ethyl-5-phenyl-1H-pyrimidin-4-on (code: DK-4); 4- {2- [2- (4-hydroxy-3-methoxyphenyl) -vinyl] -6-ethyl-4-oxo-5-phenyl-4H-pyrimidin-1-yl} - benzosulfamide (code: DK-5) . The studied objects were administered per os at a dose of 100 mg / kg (the effective dose was established in previous studies) for 7 days after 30 minutes traumatic effect on the rat's brain. On the 8th day of the study, animals were decapitated and

a biomaterial (brain, blood) was collected for subsequent assessment of changes in mitochondrial function.

Model of chronic traumatic encephalopathy

CTE in rats was modeled by repeated exposure to a 150 g load dropped from a height of 50 cm onto the parietal region of the animal cranium. The injury was reproduced once a day, every day for 7 days [10].

Biomaterial sampling and sample preparation

Rat brain, were used as biomaterial. The animals were decapitated under chloral hydrate anesthesia (350 mg / kg), organs were collected, after which the biomaterial was homogenized in a Potter mechanical homogenizer in a selection medium (1 mmol EDTA, 215 mmol mannitol, 75 mmol sucrose, 0.1% BSA solution, 20 mmol HEPES, with a pH of 7.2). The cell population was obtained by differential centrifugation, for which the obtained biogenic homogenate was centrifuged in the mode of 1.400g →3 min. at 40°C, after which the supernatant was transferred to 2 ml tubes. Next, the resulting supernatant was centrifuged at 13000g →10 min and the supernatant (culture contains native mitochondria) was removed for analysis [11]. Blood serum for determining the content of lactic acid was obtained by centrifuging (3500 RPM, 10 min.) of citrated blood.

Respirometric analysis

Analysis of the state of the respiratory function of mitochondria was carried out by the method of respirometry using the AKPM1-01L laboratory respirometer system (Alfa Bassens, Russia). The mitochondrial respiratory function was assessed by the change in oxygen consumption in the medium against the introduction of mitochondrial respiratory uncouplers. The last in the work were: oligomycin 1 μg / ml; 4 - (trifluoromethoxy) phenyl) hydrazono) malononitrile (FCCP-1 μM); rotenone - 1 μM; sodium azide - 20 mmol. The oxidation substrates were: glucose - 15 mmol. The overall assessment of mitochondrial function was determined by the level of oxygen consumption in the medium after sequential addition of oligomycin, FCCP and rotenone to the medium, and the ATP-generating ability was determined (by the difference in oxygen consumption after the addition of FCCP and oligomycin); the maximum level of respiration (according to the difference in oxygen consumption after the addition of FCCP and rotenone) and the respiratory capacity (according to the difference in oxygen consumption after the addition of FCCP and the basal level of oxygen consumption). The activity of glycolysis processes was evaluated when glucose was used as an oxidation substrate during the registration of oxygen consumption under the conditions of sequential addition of glucose, oligomycin and sodium azide to the medium. The intensity of glycolysis was determined (according to the difference in oxygen consumption after adding glucose and the basal level of oxygen consumption), glycolytic capacity (according to the difference in oxygen consumption after adding oligomycin and glucose) and glycolytic reserve (according to the difference in oxygen consumption after adding glucose and sodium azide) [12].

Methods for assessing of lactic acid concentration

The concentration of lactate was determined in the enzymatic reaction with the formation of quininomine, the concentration of which is proportional to the content of lactic acid in the sample. Incubation medium: phosphate buffer (pH 6.8), Pipes 50 mmol / L, 4-chlorophenol 6 mmol / L, 4-AAP 0.4 mmol / l, 2000 U / L lactoxydase, U / L peroxidase. The volume of the test sample is 10 μl. Sampling was carried out at 500 nm. Calculation of lactic acid was carried out according to the formula: C = Ex / E0 * 3.34 μmol / L, where Ex - absorbance of the test sample; E0 - absorbance calibration sample.

ELISA - study

The content of β-amyloid was evaluated by ELISA in the supernatant of the rat brain. We used species-specific Cloud clone reagent kit. The sample preparation and the course of the analysis corresponded to the instructions attached to the kit.

Statistical methods

Statistical processing of the data was carried out using the software package "STATISTICA 6.0" (StatSoft, USA). The results were presented as M (median value) ± SEM. To compare the groups of means, one-factor variant of ANOVA with the Newman-Keuls post-test was used. Differences were considered statistically significant at $p < 0.05$.

Results

During the study, it was found that the NC group of animals compared to rats of the intact group showed a decrease in ATP-generating activity, the maximum level of respiration and respiratory capacity (Fig. 1) by 8.1 times (p <0.05); 26.8 times (p <0.05) and 14.1 times (p <0.05), respectively. In addition, animals deprived of pharmacological support under conditions of CTE showed an increase in anaerobic metabolism, as evidenced by an increase in glycolysis intensity in this group of rats relative to intact animals by 3 times (p <0.05), as well as a decrease in glycolytic capacity and glycolytic reserve by 46.1 times (p <0.05) and 13.6 times (p <0.05), respectively (Fig. 2). At the same time, the content of lactate (Fig. 3) in serum and β-amyloid (Fig. 4 a) in brain tissue in rats without pharmacological support increased relative to intact groups animals in 3.6 times (p <0.05) and 25.3 times (p <0.05), respectively. The use of compound DK-1 contributed to an increase in ATP-generating activity, the maximum level of respiration and respiratory capacity (Fig. 1) compared to the NC group of rats by 4.4 times (p <0.05); 8.6 times (p <0.05) and 2.9 times (p <0.05), respectively. At the same time, in the group of rats receiving the compound DK-1 relative to the NC of the group of rats, the intensity of glycolysis decreased by 38.2% (p <0.05), and the opposite glycolytic capacity and glycolytic reserve increased 7.2 times (p <0, 05) and 13.6 times (p <0.05), respectively (Fig. 2). In addition, the use of the compound DK-1 contributed to a decrease in the concentration of lactic acid (Fig. 3) and β-amyloid (Fig.4) by 60.1% (p <0.05) and 2.4 times (p <0.05) regarding the NC group. Against the background of the introduction of a compound under the code DK-2 to animals, an increase in the ATP-generating activity, the maximum level of respiration and respiratory capacity (Fig. 1) was observed by 2.4 times (p <0.05); 3.4 times (p <0.05) and 39% (p <0.05), respectively, relative to the NC group of rats. Also in animals treated with the test-object DK-2, the intensity of glycolysis decreased in comparison with the NC group of animals by 36.9% (p <0.05) with an increase in glycolytic capacity and glycolytic reserve (Fig.2) by 7.2 times (p <0.05) and 4.1 times (p <0.05), which contributed to a decrease in the concentration of lactic acid (Figure 3) and β-amyloid (Figure 4) in this group of animals by 46.6% (p < 0.05) and 3.1 times (p <0.05), respectively. The use of DK-3 compound in rats showed an increase in ATP-generating activity, the maximum level of respiration and respiratory capacity (Fig. 1) compared to the NC group of rats by 7.9 times (p <0.05); 2.9 times (p <0.05) and 51% (p <0.05), respectively. In addition, in animals treated with compound DK-3 relative to the NC group of rats, a decrease in the intensity of glycolysis (Fig. 2), concentration of lactic acid (Fig. 3) and β-amyloid (Fig. 4) was observed by 33.2% (p <0.05); 49.1% (p <0.05) and 4.5 times (p <0.05), respectively. At the same time in animals that were administered compound DK-3. glycolytic capacity and glycolytic reserve exceeded the analogous parameters of the NC group of rats by 2.5 times (p <0.05) and 5.3 times (p <0.05), respectively. In animals, when using compound DK-4 relative to rats of the NC group, there was an increase in ATP-generating activity and maximum level (Fig. 1) by 2 times (p <0.05) and 3.7 times (p <0.05), respectively . Also in animals treated with compound DK-4, a decrease in glycolysis intensity was observed in relation to the NC group of rats (Fig. 2) - by 38.6% (p <0.05), the content of lactic acid (Fig. 3) - by 24 , 1% (p <0.05) and β-amyloid- by 2.1 times (p <0.05) with an increase in glycolytic capacity and glycolytic reserve by 2.5 times (p <0.05) and 4.5 times (p <0.05), respectively. The administration of test-object under DK-5 code to animals in relation to the NC group of rats showed an increase in ATP-generating activity and maximum level of respiration by 2.7 times (p <0.05) and 4.5 times (p <0.05), respectively (Fig. 1), as well as a decrease in the intensity of glycolysis (Fig. 2) - by 36.1% (p <0.05), the concentration of lactic acid (Fig. 3) - by 38.7% (p <0.05) and β-amyloid (Fig. 4) - 2.5 times (p <0.05), followed by an increase in glycolytic capacity and glycolytic reserve by 4.4 times (p <0.05) and 4.1 times (p <0.05) respectively.

Discussion

CTE is a chronic neurodegenerative disease resulting from repeated episodes of traumatic brain injury. CTE is often found in persons experiencing significant physical overload - highly skilled contact sports athletes (boxing, wrestling, American football, hockey). Cases of CTE are also reported in professional military personnel exposed to a shock wave from explosive devices [13]. At the same time, currently there are no specific methods for treating

CTE, and the available approaches to treating CTE are mainly prophylactic (using personal protective equipment), which suggests the urgency of finding new pharmacotherapeutic approaches to treating CTE [14]. In the study, it was found that the use of pyrimidine-4H-1OH derivatives under conditions of experimental CTE contributed to the restoration of mitochondrial function, which was reflected in the improvement of aerobic metabolism (ATP-generating activity, maximum respiration rate and respiratory capacity), as well as a decrease in the intensity of reactions anaerobic metabolism. At the same time, as a result of the observed metabolic shifts, animals showed a decrease in the concentration of lactic acid and β-amyloid. In a number of data are given that the correction of metabolic disturbances under the conditions of CTE is one of the most potentially effective methods for correcting this state [15]. So the restoration of mitochondrial function can contribute to the reduction of secondary cellular damage by restoring energy balance, suppressing reactions of apoptosis and oxidative stress. It has been established that the adequate functioning of mitochondria prevents the activation of proapototic proteins (Bax, caspase-3, PUMA, AIF), which favorably affects the preservation of the structural integrity of neuronal tissue [16]. In addition, the stabilization of electron transfer reactions in the mitochondrial respiratory chain can prevent the dissociation of redox reactions at the level of complexes I and II, which suppresses the generation of oxygen free radicals and oxidative stress [17]. Thus, the restoration of mitochondrial function in CTE conditions can contribute to the elimination of the three leading secondary pathogenetic mechanisms of neuronal damage - energy deficiency, apoptosis and oxidative stress, which makes mitochondrial dysfunction correcting compounds promising for treatment of CTE [18].

Conclusion

In the course of this study, it was found that the use of new pyrimidine-4H-1OH derivatives under CTE conditions contributed to the restoration of mitochondrial function, expressed in an increase in ATP-generating activity, maximum respiration, respiratory capacity, glycolytic capacity and glycolytic reserve, as well as a decrease in glycolysis intensity. At the same time, as a result of these changes, a decrease in the concentration of lactic acid and β-amyloid was noted in rats, on the basis of which it can be assumed that pyrimidin-4H-1OH derivatives are promising as a means of correcting CTE with metabolic effects.

Acknowledgements And Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflicts Of Interest

The authors statement no conflict of interest with the submitted manuscript.

References

1. Stein TD, Alvarez VE, McKee AC. Concussion in Chronic Traumatic Encephalopathy. Curr Pain Headache Rep. 2015;19(10):47.
2. Montenigro PH, Baugh CM, Daneshvar DH, et al. Clinical subtypes of chronic traumatic encephalopathy: literature review and proposed research diagnostic criteria for traumatic encephalopathy syndrome. Alzheimers Res Ther. 2014;6(5):68.
3. Baugh CM, Stamm JM, Riley DO, Gavett BE, Shenton ME, Lin A, et.al. Chronic traumatic encephalopathy: neurodegeneration following repetitive concussive and subconcussive brain trauma. Brain Imaging Behav. 2012;6:244–254.
4. Stein TD, Alvarez VE, McKee AC. Chronic traumatic encephalopathy: a spectrum of neuropathological changes following repetitive brain trauma in athletes and military personnel. Alzheimers Res Ther. 2014;6(1):4.
5. Hay J, Johnson VE, Smith DH, Stewart W. Chronic Traumatic Encephalopathy: The Neuropathological Legacy of Traumatic Brain Injury. Annu Rev Pathol. 2016;11:21–45.
6. Suliman HB, Piantadosi CA. Mitochondrial Quality Control as a Therapeutic Target. Pharm.rev. 2016;68:20–48.
7. Grimm A, Eckert A. Brain aging and neurodegeneration: from a mitochondrial point of view. J Neurochem. 2017;143(4):418–431.
8. Ranftler C, Meisslitzer-Ruppitsch C, Neumüller J, Ellinger A, Pavelka M. Golgi apparatus dis- and reorganizations studied with the aid of 2-deoxy-D-glucose and visualized by 3D-electron

tomography. Histochem Cell Biol. 2017;147(4):415–438.

9. Chen GF, Xu TH, Yan Y, et al. Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacol Sin. 2017;38(9):1205–1235.

10. Wallis A, Ball M, McKechnie S, Butt H, Lewis DP, Bruck D. Examining clinical similarities between myalgic encephalomyelitis/chronic fatigue syndrome and D-lactic acidosis: a systematic review. J Transl Med. 2017;15(1):129.

11. Turner RC, Lucke-Wold BP, Logsdon AF, et al. Modeling Chronic Traumatic Encephalopathy: The Way Forward for Future Discovery. Front Neurol. 2015;6:223.

12. Patel SP, Sullivan PG, Pandya JD, et al. N-acetylcysteine amide preserves mitochondrial bioenergetics and improves functional recovery following spinal trauma. Exp Neurol. 2014;257:95-105

13. Lesnefsky EJ, Chen Q, Hoppel CL. Mitochondrial Metabolism in Aging Heart. Circ Res. 2016;118(10):1593-611.

14. Armstrong RA, McKee AC, Stein TD, Alvarez VE, Cairns NJ. A quantitative study of tau pathology in 11 cases of chronic traumatic encephalopathy. Neuropathol Appl Neurobiol. 2017;43(2):154–166.

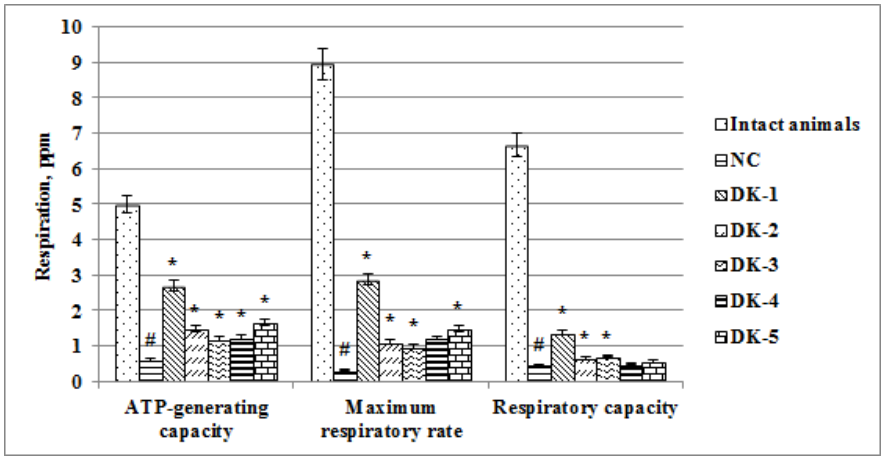
15. Saulle M, Greenwald BD. Chronic traumatic encephalopathy: a review. Rehabil Res Pract. 2012;2012:816069.

16. Huang YN, Yang LY, Greig NH, Wang YC, Lai CC, Wang JY. Neuroprotective effects of pifithrin-α against traumatic brain injury in the striatum through suppression of neuroinflammation, oxidative stress, autophagy, and apoptosis. Sci Rep. 2018;8(1):2368.

17. Marine JC. Keeping p53 in check: essential and synergistic functions of Mdm2 and Mdm4. Cell D. Diff. 2006;13:927–934.

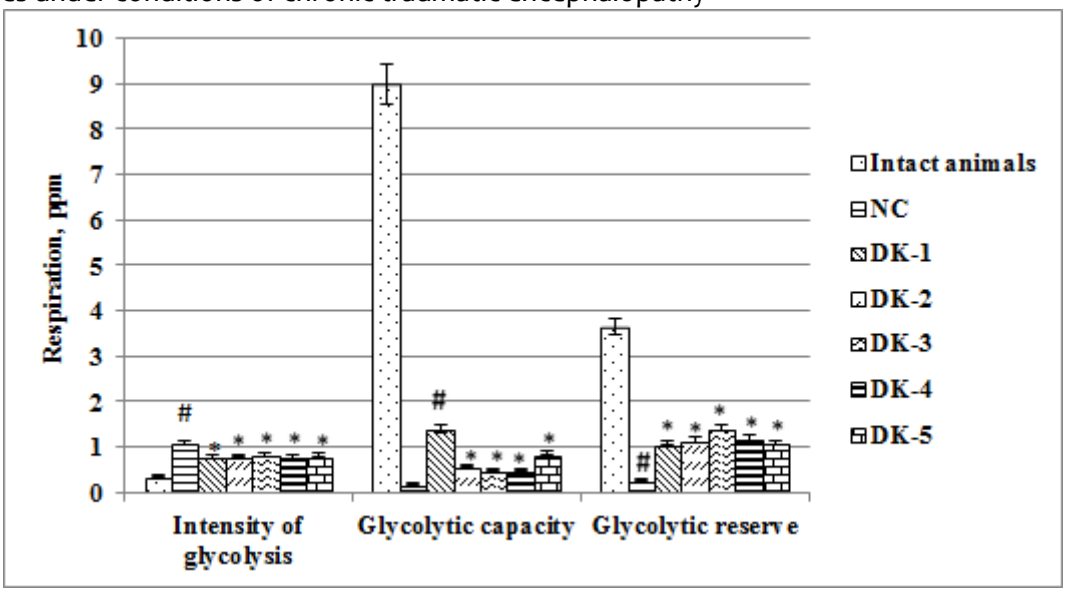
18. Bains M, Hall ED. Antioxidant therapies in traumatic brain and spinal cord injury. Bioch. et Biophy.acta. 2012;1822:675–684.

Figure 1. The effect of the test-compounds on the change in the overall respiratory function of mitochondria in conditions of chronic traumatic encephalopathy



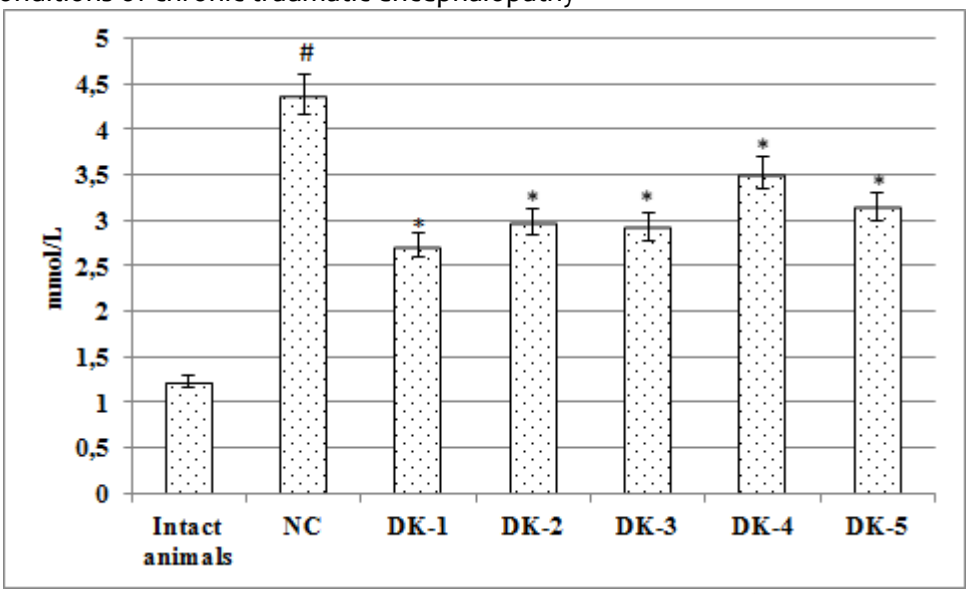
Note: # - statistically significant relative to the intact animals ($p < 0.05$, Newman-Keuls test);
* - statistically significant relative to the NC group of animals ($p < 0.05$, Newman-Keuls test).

Figure 2. The effect of the test-compounds on the change in the activity of anaerobic mitochondrial processes under conditions of chronic traumatic encephalopathy



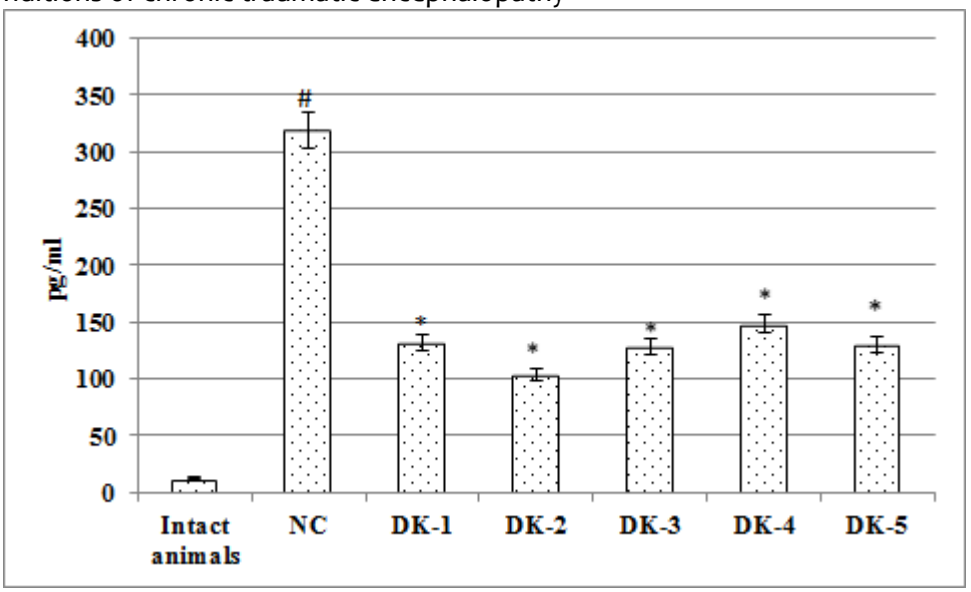
Note: # - statistically significant relative to the intact animals ($p < 0.05$, Newman-Keuls test);
* - statistically significant relative to the NC group of animals ($p < 0.05$, Newman-Keuls test).

Figure 3. The effect of the test-compounds on the change of the concentration of lactic acid in serum under conditions of chronic traumatic encephalopathy



Note: # - statistically significant relative to the intact animals (p <0.05, Newman-Keuls test);
***** - statistically significant relative to the NC group of animals (p <0.05, Newman-Keuls test).

Figure 4. The effect of the test- compounds on the change in the concentration of β-amyloid in the brain tissue of rats in conditions of chronic traumatic encephalopathy



Note: # - statistically significant relative to the intact animals (p <0.05, Newman-Keuls test);
***** - statistically significant relative to the NC group of animals (p <0.05, Newman-Keuls test).