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TADALAFIL AS AN AGENT OF PHARMACOLOGICAL PRECONDITIONING IN ISCHEMIC – REPERFUSION BRAIN INJURY

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Abstract

Introduction: Ischemic stroke or cerebral infarction is the main pathology among severe forms of vascular lesions of the brain. One of the more effective non-medicamental methods of treatment is pharmacological preconditioning. Pharmacological neuroprotection is one of the treatment areas to reduce the damage in ischemic stroke and other modifications of brain ischemia. Therefore, the development and introduction of new pharmacological agents that can reduce the degree of ischemic-reperfusion injury of the brain, remains one of the major challenges of modern medicine. The most promising to explore, from our point of view, is a PDE-5 inhibitor.

Goal: Improving the efficiency of pharmacological cerebroprotective with pharmacological preconditioning of the PDE-5 inhibitor (tadalafil) in comparison with recombinant erythropoietin («Epocrin») and a neuroprotectant "Gliatilin".

Materials and methods: In the pilot study used an integrated approach to the study of neuroprotective effects of pharmacological preconditioning in animals with ischemia-reperfusion brain damage in four-vascular total ischemia of the brain. In the complex of methods included evaluation of neurological deficit, behavioral status, level of markers of brain damage S100b and NSE, morphometry. To compare the efficacy of tadalafil (1 mg/kg) in the experiment used recombinant erythropoietin «Epocrin» (50 IU/kg) and the neuroprotectant "Gliatilin" (85.7 mg/kg).

Results and discussion: Prophylactic intraperitoneal administration of PDE-5 inhibitor, tadalafil (1 mg/kg) exerted cerebroprotective effect in modeling of ischemia-reperfusion, expressed in reducing the severity of neurological deficit (0.8±0.21 points), compared with the control group (of 2.05±0.49 points); increase in the number of stands at 1.7 times and 2.2 hanging times; not a big increase in overall activity, patterns of movement, maximum speed, total distance increased 1.5 times, decrease rest time by 1.2 times; the reduction in the concentration of damage markers S100b 3.5 times and the NSE in 2 times. A number of distinctive characteristics the morphometric study, as well as a set of symptoms, manifestations of behavioral reactions confirm the fact of cerebroprotective properties of tadalafil in comparison with the control group animals.

Conclusions: the conducted research showed cerebroprotective property of an inhibitor of phosphodiesterase type 5, tadalafil. The results of the study clearly indicate the prospects of its use in vascular pathology of the brain.

Key words: pharmacological preconditioning, ischemia of the brain, tadalafil.

Introduction

Ischemic stroke or cerebral infarction is the main pathology among severe forms of vascular lesions of the brain [1, 2]. In Russia, this pathology ranks first as the cause of disability, and in the structure of total mortality is second [3]. The problem of timely pathogenetic treatment of this pathology is crucial due to the widespread prevalence, high mortality, disability and social exclusion suffered its patients [4]. To date, there are

two approaches to solve this problem: the use of medication (pharmacological) medications and non-pharmacological methods affecting the mobilization of domestic genetically-determined protective mechanisms, evolutionary acquired. With therapy achieves the restoration of blood flow in the ischemic region. Such therapies include: the destruction of blood clots, anticoagulant and antiplatelet therapy and hemodilution (using low molecular weight dextrans). To date, there is a need for search and development of new effective cerebroprotectors that could improve the course of disease, prevent the development of neurodegenerative processes in the brain, it is more useful to provide emergency assistance, reduce mortality, reduce the duration of the acute period, to reduce the disabling effects of the disease. One of the more effective non-medicamental methods is pharmacological preconditioning [5, 6]. Pharmacological neuroprotection is one of the treatment areas to reduce the damage in ischemic stroke and other modifications of brain ischemia. Therefore, the development and introduction of new pharmacological agents that can reduce the degree of ischemic-reperfusion injury of the brain, remains one of the major challenges of modern medicine. The most promising to explore, from our point of view, is a PDE-5 inhibitor.

Currently in the neuro emergency care requires a constant introduction of new methods for the early detection and prevention of secondary damaging factors, including and stroke. The latest guidelines of American Heart Association / American Stroke Association and the Institute of cerebrovascular pathology and stroke are not the proposed methods of predicting the course of AI. According to a leading international clinical recommendations on the treatment and prevention of the consequences of stroke and to data obtained by the majority of large randomized trials to study the efficacy of cerebroprotective drugs, there was no neuroprotective regimen of drugs, which showed significant improvement of outcome of stroke. Now known for a large number of mechanisms that forms the basis preconditioning: open launcher agents, substances – mediators, a number of final targets [5, 6, 7]. One of the many branches of the study of agents that have a pharmacological preconditioning is the impact on the path of NO – cGMP – protein kinase G (PK – G) [8]. Recently, a new potential therapeutic strategy for inhibitor of phosphodiesterase type 5 that it has a protective effect on the brain neurogenic, neurodegenerative diseases and memory loss [9, 10, 11]. Currently, no drugs based on iPDE – 5 approved for clinical use in stroke, therefore, it seems appropriate to study the presence of cerebroprotective properties of iPDE – 5 and their effectiveness.

Goal: Improving the efficiency of pharmacological cerebroprotective with pharmacological preconditioning of the PDE-5 inhibitor (tadalafil) in comparison with recombinant erythropoietin («Epocrin») and a neuroprotectant "Gliatilin".

Materials and methods

The study was performed on 410 weinbrenner adult male rats line "Wistar" 5-6 months of age weighing 220-250 g. the animals comply with all the rules of good laboratory practice in preclinical studies in Russia. Animals were kept under standard conditions, corresponding to sanitary regulations (No. 1045-73), approved by the USSR health Ministry 06.04.73 on the device, equipment and maintenance of experimental biological clinics (vivariums) and GOST R 53434-2009. Vivisection was carried out according to ethical principles for the treatment of laboratory animals "of the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No.123".

Methods of research in vivo. All the experiments were performed in accordance with methodological recommendations on preclinical study of drugs for treatment of disorders of cerebral circulation and migraine [1, 4]. In this study we used several methods reproduce the experimental models of cerebral ischemia: the "two-vascular " and " four-vascular ". Ischemic stroke ischemic was simulated by a temporary occlusion of two common carotid arteries with subsequent reperfusion or by coagulation of two vertebral carotid artery with temporary occlusion of two common carotid arteries with subsequent reperfusion. Depending on the purpose of the study the duration of episodes of ischemia-reperfusion can vary [12]. Evaluation of adequacy of implementation of occlusion of the arteries supplying the brain, was carried out using the recording of the electrical activity of the brain of the animal and the level of microcirculation in the

sclera of the eye using laser Doppler flowmetry on the device Biopac Systems Inc. MP150 EEG100C and LDF 100C. The methods were implemented using the software AcqKnowledge 4.2. The criterion for correct execution of the techniques was the reduction of EEG amplitude and a decrease in the level of the microcirculation [13, 14, 15]. To assess the neurological status of rats used several methods:

1. Point scale of evaluation of the McGraw stroke in the modification of I. V. Gannushkina [13, 16]. Within the group of rats with signs of neurological deficit were divided into animals with mild, moderate and severe symptoms of neurological deficit. If the animal has several signs of neurological deficit, the scores are summed.

2. "Elevated cross maze" behavioral test to examine the activity, emotional state and level of anxiety in laboratory animals during the experiment [17]. In the experiment, was used to install the labyrinth of the firm Panlab Harvard Apparaturs LE 846.

3. "Infrared activity monitor" – IR Actimeter allows you to test arbitrary locomotor activity, numbers and duration of episodes of getting up on his hind legs, stereotypical movements and exploratory behavior in the model of "perforated field" in terms of day and night illumination [17]. Is used to assess exploratory behavior. Were used in the experiment installation firm Panlab Harvard Apparaturs LE 8825.

Methods in vitro. For more accurate results and correct interpretation of animal plasma is examined for two markers of brain damage – S100b and NSE. Determined by their concentration in the serum [18]. Morphometric study of histological [19, 20, 21] of brain slices was performed under a microscope MIKMED-6 (LOMO, Russia). The Protocol of the study. Justification of doses. At the beginning of the experiment, all animals were randomized according to the degree of resistance to hypoxia. The experiment involved animals with average resistance. Under the objectives of the study, we have developed a model of pathology. For this simulated local two-vascular IGM group of rats (n = 10) and total IGM four-vascular group of rats (n = 10). As the comparison drugs used recombinant erythropoietin «Epocrin» and neuroprotectant "Gliatilin". Tadalafil (Eli Lilly Vostok S. A., Switzerland) were administered once intragastrically at a dose of 1 mg/kg for 60 min prior to the ischemia simulation. The selected dose corresponds to an average therapeutic dose for humans calculated by the formula interspecies transfer [8]. «Epocrin» – recombinant erythropoietin (FGUP GosNII OCHB, Saint-Petersburg, Russia) was administered once at a dose of 50 IU/kg intraperitoneally 30 minutes before the modeling of the IGM. The dose chosen in order to avoid stimulation of erythropoiesis and the presence of the protective effect is proven in many organs and tissues [22, 23, 24, 25, 26, 27]. "Gliatilin" (ITALFARMACO, S. p.A. Italy) was injected once intragastrically at a dose of 85.7 mg/kg in the first case, for 30 min prior to simulation of ischemia, the second after 30 min after. The selected dose corresponds to the dose calculated by the formula interspecies transfer of doses. Glibenclamide is a blocker of ATP-sensitive potassium channels ("Maninil", Berlin-Chemie AG), was injected once intragastrically at a dose of 5 mg/kg over 60 min. before simulation of the IGM [29].

The study Protocol included the following stages: modeling cerebral ischemia; evaluation of the level of the electroencephalogram of an animal and microcirculation by LDF; assessment of behavioral status (2 d) and neurological deficits at 1, 3, 7 and 14 days after modelling pathology; determining the presence of specific markers of brain damage S100b and NSE; removal of animals from the experiment and the taking of material for morphometric studies, analysis of blood for the presence of markers of brain damage S100bи NSE. In the Protocol of study of protective action of iPDE -5, EPO and "Gliatilin" included the following groups of animals (each group 10 animals):

1. The group of intact animals.
2. The group of false-operated animals.
3. Group two-vascular model 4-min.
4. Group four-vascular model 3-min.
5. Group four-vascular model 4-min.
6. Group four-vascular model for 4,5-min.
7. Group four-vascular model 4-min + iFDE-5.
8. Group four-vascular model 4-min + EPO.
9. Group four-vascular model 4-min + "Gliatilin" (CI).
10. Group four-vascular model 4-min +"Gliatilin" (after CI).
11. Group four-vascular model 4-min + iFDE-5+ "Gliatilin".

12. Group glib + iFDE-5 + four-vascular model 4-min.

13. Group glib+ EPO + four-vascular model 4-min.

For all of the data was used descriptive statistics. The received data is checked for normality of distribution. Using the Shapiro-Wilk test have chosen the type of distribution. In the case of the normal distribution was calculated the average value M and standard error of the mean m. In cases of abnormal distribution were calculated median and quartile Me the scope of QR. Intergroup differences were analyzed by parametric (student's t-test) or nonparametric (Mann-Whitney test) methods, depending on the distribution type. Differences were determined at 0,05 level of significance. Statistical analysis was performed using software Statistica 10.0 [30, 32, 33].

Results and discussion

In the beginning of the experiment was developed a set of methods for quantitative evaluation of disorders in ischemic-reperfusion brain injury, as described earlier. The true criterion of the performed techniques was to reduce the amplitude of the EEG and the level of microcirculation in the sclera of the eye by LDF. Also was selected as the optimal model of pathology – four-vascular and experimentally matched the duration of the ischemic period of 4 min. This model of pathology was characterized by the development of neurological deficits (of 2.05±0.49 points); reduction of the number of racks in 2 times and hanging 2.7 times roll "Elevated cross maze"; violation of behavioral status in the test of actimetry, which was manifested in the decline in overall activity in 2 times, reducing the number of patterns of movement in 2 times, reduce the maximum speed by 1.5 times, decrease of passing the total distance of 2.3 times the increase in leisure time in 1.7 times; the increase in the concentration of damage markers S100b 2.5 times and the NSE in 2 times; morphological changes: the presence of 90% hyperchromic neurons in the frontal lobes and the hippocampus. Depending on the research method for the control data received intact or false-operated animals.

Cerebroprotective effects of PDE-5 inhibitor, tadalafil. Neurological deficit in rats on the background correction pharmacological preconditioning tadalafil (1 mg/kg) was more mild symptoms compared to the group of animals with CI without drug administration. 3, 7, 14 days was still polutes right eye. In behavioral test, ECM group of rats with CI and correction tadalafil manifested itself more actively in comparison with the control group. When comparing the locomotor activity of animals in the control group with the group in the correction tadalafil in the test of actimetry for infrared activity monitor IR Actimeter the activity of rats with pre-administration of the drug is higher (figure 1).

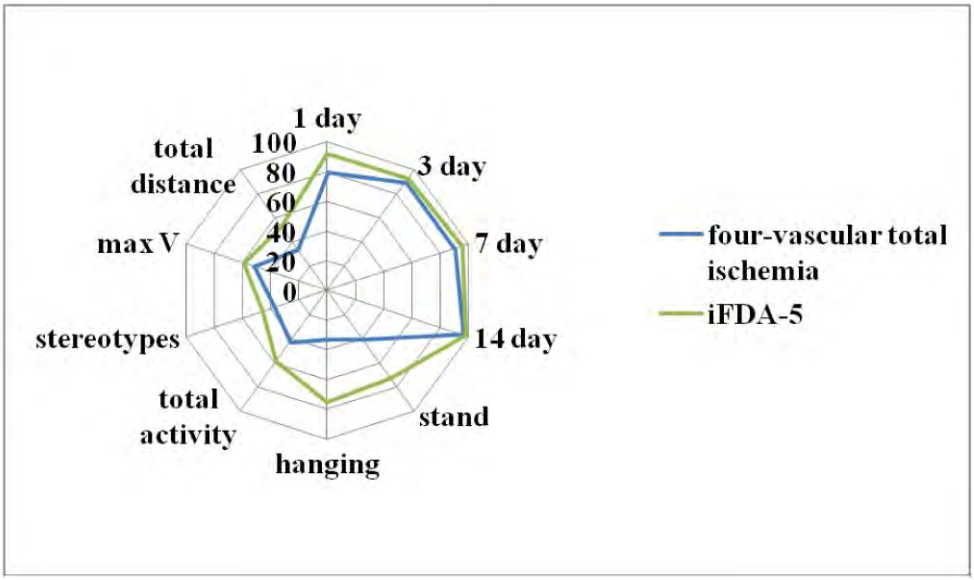

Fig. 1. Effect of tadalafil on indicators of activity of the animals in the modeling of cerebral ischemia (iFDE-5)

When analyzing the level of S100b and NSE in animals of the group with CI, with a

preliminary correction tadalafil observed a decrease in the levels of markers of damage even below the level of the control group (figure 2).

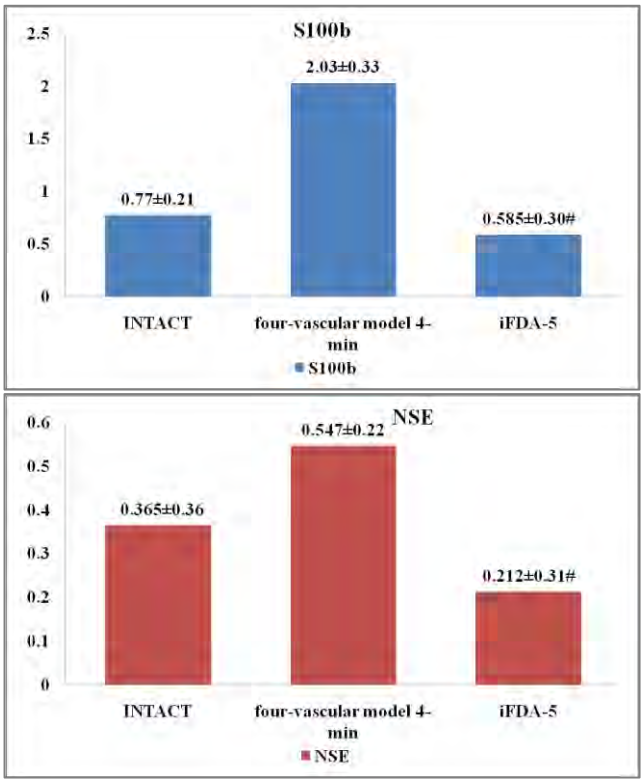

Fig. 2. Effect of tadalafil on the concentration of markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) (M ± m; n = 10). Note – * p<0.05, # – p < 0.05 in relation to intact rats

In brain slices of rats with a preliminary introduction tadalalafil observed: hyperchromia neurons in the frontal lobes 43.4% and in the area of the hippocampus 90%; hypochromia neurons in the frontal lobes of 43.4% (in the area of the hypochromic hippocampal neurons were absent); neurons with two ravines in the frontal lobes of 13.2% and in the area of the hippocampus 10%. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figure 3).

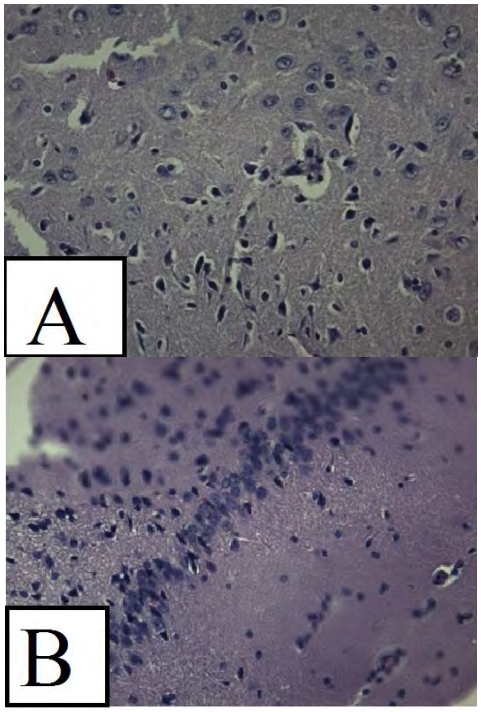

Fig. 3. Brain Slices rat CI and prior to the introduction of tadalafil
A- the frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus X 400 Deposit. hematoxylin+eosin

Cerebroprotective effects of recombinant erythropoietin «Epocrin». Neurological deficit in rats with pre-introduction "Epocrin" (50 IU/kg) were with mild symptoms, compared to control group animals. 3, 7, 14 day remained the floor ptosis right eye. In behavioral test, ECM group of rats with correction "Epocrin" proved to be active. When comparing the locomotor activity of animals in the control group with the group in the correction of "Apocrine" in the test of actimetry for infrared activity monitor IR Actimeter the activity of the rats with pre-infusion is much higher relative to the control group (figure 4).

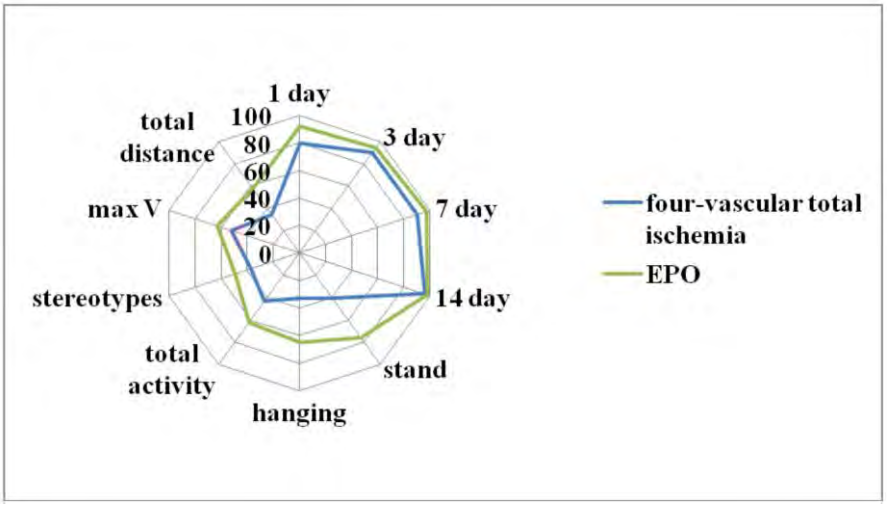

Fig. 4. The Effect of recombinant erythropoietin («Epocrin») on indicators of activity of animals in modeling cerebral ischemia

When analyzing the level of S100b and NSE in animals of the group with CI, with a preliminary introduction of "Epocrin" observed the decrease in the concentration of markers of damage below the level of intact animals ($p > 0.05$) (figure 5).

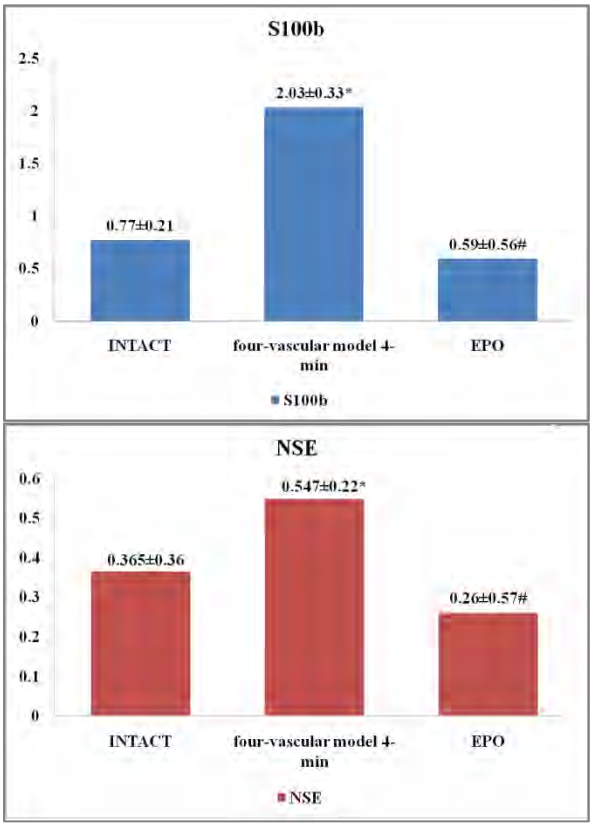

Fig. 5. Effect of recombinant erythropoietin («Epocrin») on markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) (M ± m; n = 10).
Note – * $p<0.05$, # – $p < 0.05$ in relation to intact rats

In brain slices of rats with a pre-introduction "Epocrine" was also observed by hyperchromia neurons is 43.3% in the frontal lobes and 69.7% in the area of the hippocampus; neurons hypochromia – 43.4% in the frontal lobes and 20.3% in the area of the hippocampus; neurons with two ravines neurons and 13.3% in the frontal lobes and 10% in the area of the hippocampus. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figure 6).

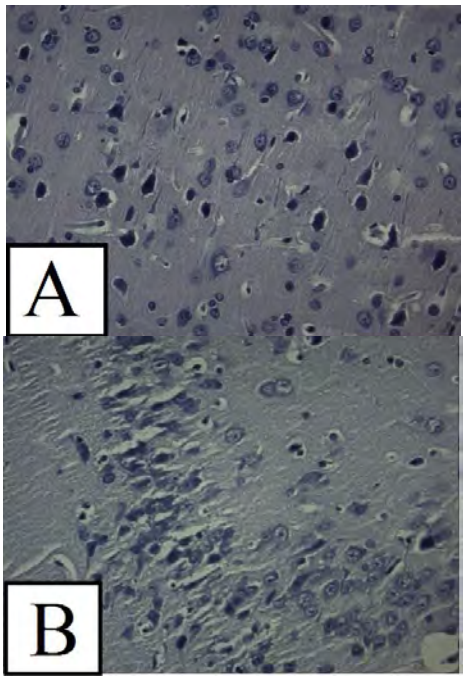

Fig. 6. Brain Slices rat IGM and prior to the introduction of "Epocrin":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus X 400 Deposit. hematoxylin+eosin

Cerebroprotective effects of the neuroprotectant "Gliatilin". Neurological deficit of the groups of rats as a prophylactic and therapeutic introduction "Gliatilin" (85.7 mg/kg) was with a medium degree of gravity. 3, 7, 14 days was preserved paralysis of hind left limb and remained the floor ptosis, ptosis of the right eye. The group of rats with the introduction of the drug for medicinal purposes in the modeling of the pathology observed the same symptoms of neurological deficit except Manege movements. Visually the activity of rats in these experimental groups did not differ from the control group as in the test ECM and test altimetry (figure 7).

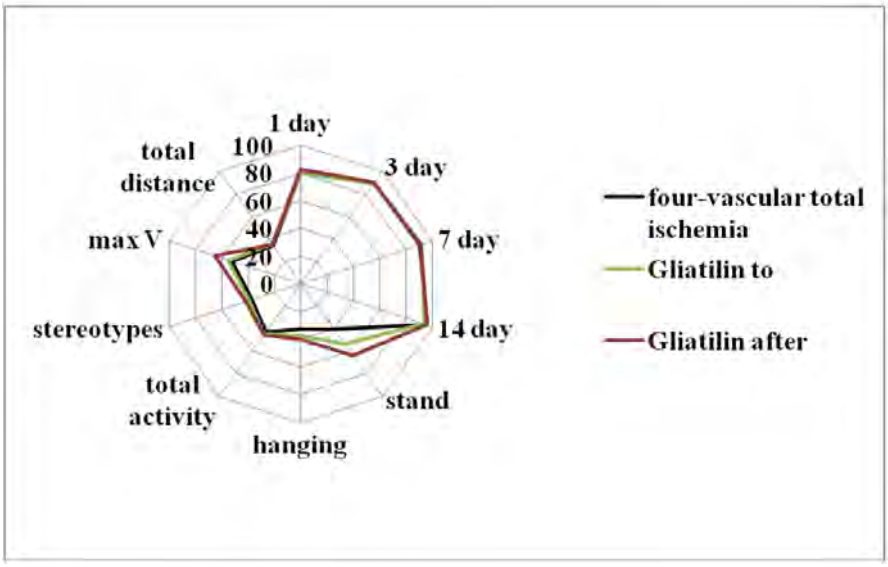

Fig. 7. Influence of neuroprotectant "Gliatilin" on indicators of activity of animals in modeling cerebral ischemia

The level of S100b and NSE in the animals of group a prophylactic introduction of "Gliatilin" above the level of the control group. The level of markers of damage a group of rats with a therapeutic drug is lower than in the control group (figure 8).

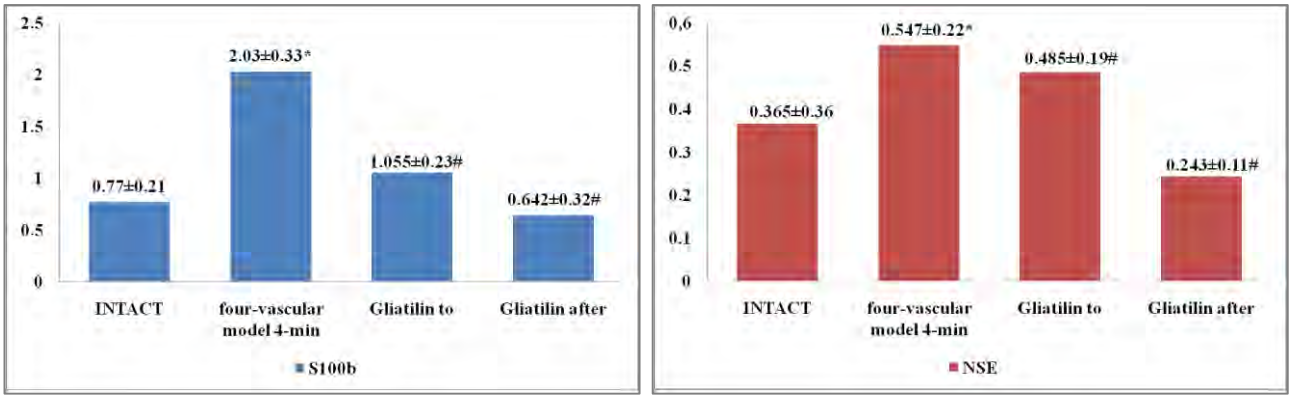

Fig. 8. Influence of neuroprotectant "Gliatilin" markers of brain damage in the plasma of animals in 3 days (S100b-µg/l; NSE – ng/ml) (M ± m; n = 10).
Note – * – p<0.05, # – p < 0.05 in relation to intact rats

In brain slices of rats in the correction of "Gliatilin" both preventive and therapeutic purposes observed: hyperchromia neurons were 92% and 88% in the frontal lobes, 89% and 84% in the area of the hippocampus; neurons hypochromia – 8% and 12% in the frontal lobes, in the area of the hypochromic hippocampal neurons were absent; neurons with two ravines neurons in the frontal lobes absent, in the area of the hippocampus, their number corresponded to 11% and 16%. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figures 9, 10).

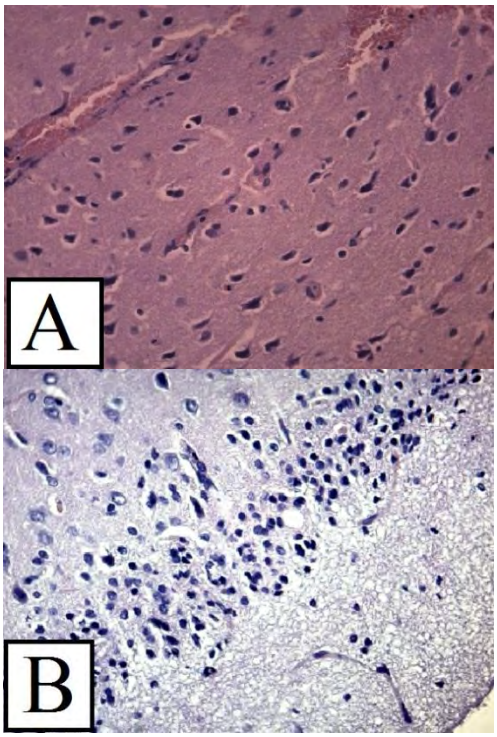

Fig. 9. Brain Slices of a rat a prophylactic introduction of "Gliatilin":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus X 400,. hematoxylin+eosin

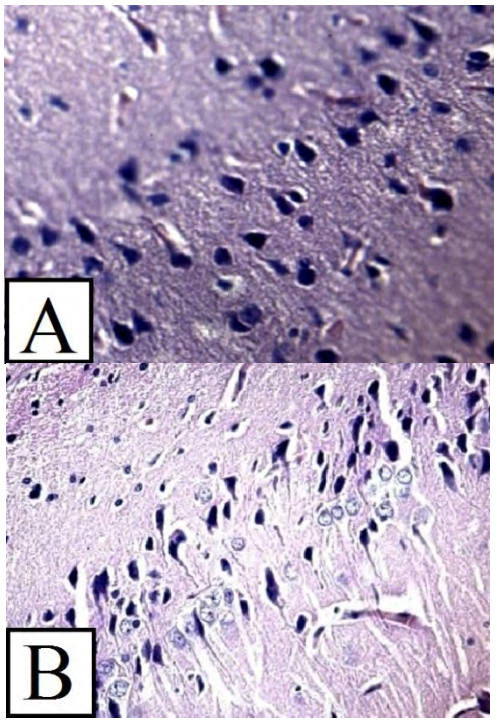

Fig. 10. Brain Slices of the rat with a medical introduction of the "Gliatilin":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus, X 400, hematoxylin+eosin

Additive effect of combined use of PDE-5 inhibitor, tadalafil and neuroprotectant "Gliatilin". Neurological deficit in rats, "iFDE-5+CI+Gliatilin" (1 mg/kg; 85,7 mg/kg) were with mild symptoms. 3, 7, 14 days was preserved the floor ptosis right eye. In behavioral test, ECM group of rats", iFDE-5+CI+Gliatilin" were active, compared with the group CI. Compared with groups of rats in monotherapy, this group has a significantly greater activity. Locomotor activity of rats ", iFDE-5+CI+Gliatilin" in the test of actimetry for infrared activity monitor IR Actimeter higher than in rats with BL a group of rats in monotherapy (figure 11).

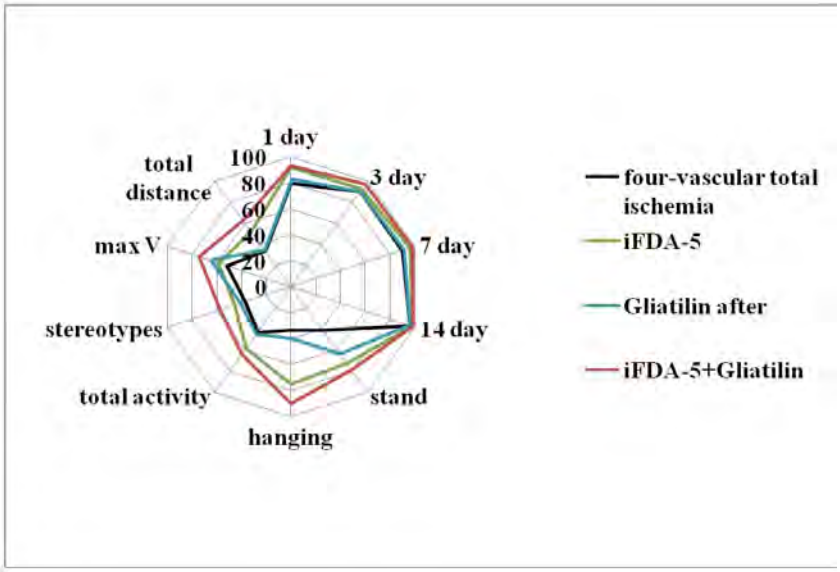

Fig. 11. The Effect of combination of tadalafil (iPDE -5)and neuroprotectant "Gliatilin" on indicators of activity of animals in modeling cerebral ischemia

When analyzing the level of S100b and NSE in the animals of group " iPDE -5+IGM+Gliatilin" observed the decrease in the concentration of markers of damage below the level of intact animals ($p > 0.05$) (figure 12).

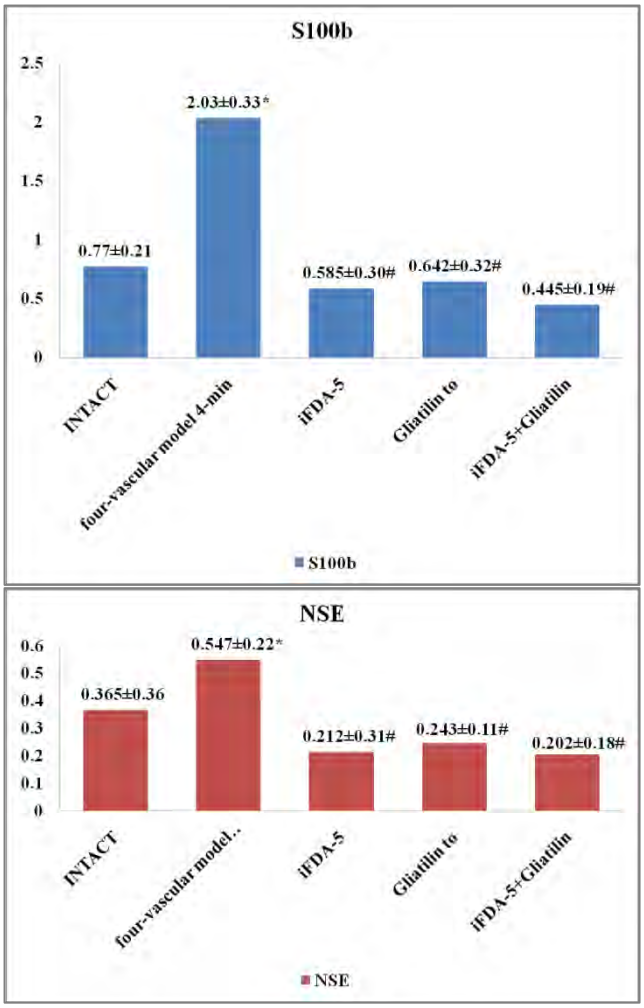

Fig. 12. Effect of combination of tadalafil (iPDE-5) and neuroprotectant "Gliatilin" markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) (M ± m; n =10). Note – * $p<0.05$, # – $p < 0.05$ in relation to intact rats

In brain slices of rats ", iFDE-5+CI+Gliatilin" observed: hyperchromia neurons and 32% in the frontal lobes and 70% in the area of the hippocampus; hypochromia neurons is 54.8% in the frontal lobes and 15% in the area of the hippocampus; neurons with two ravines neurons of 13.2% in the frontal lobes and 15% in the area of the hippocampus. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figure 13).

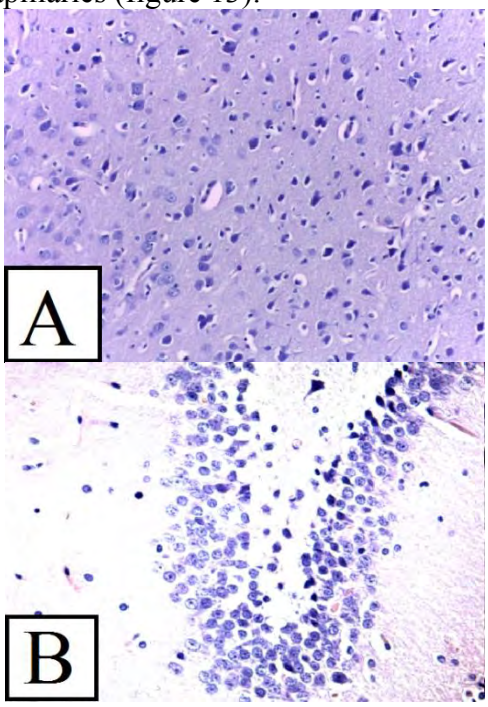

Fig. 13. The brain Slices of the rat group "iPDE-5+IGM+Gliatilin":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus, X 400, hematoxylin+eosin

The contribution of K+ATP-channels to the implementation of the cerebroprotective effects of pharmacological preconditioning. Neurological deficit in animal groups "Glib + iFDE-5 + CI" and "Gleb + EPO + CI" (5 mg/kg) were medium severity, which was not statistically different from the group with only IGM. 3, 7, 14 days was preserved paralysis of left rear leg and the floor ptosis /ptosis of the right eye. In the behavioral test ECM behavior of groups of rats "Glib + iFDE-5 + CI", "Glib + EPO + CI" was characterized by decreased horizontal activity, a significant reduction in vertical activity. The activity of rats between these experimental groups are similar, which confirms the cancellation preconditioning properties of drugs due to prior administration of blocker of K+ATP channels (figure 14).

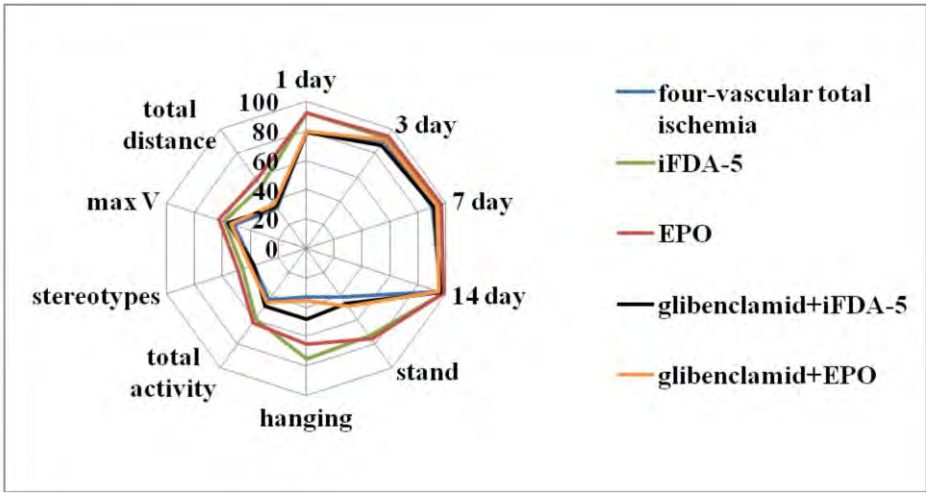

Fig. 14. Effect of tadalafil (iPDE-5), "Epocrin" with the prior introduction of glibenclamid on the activity of animals in modeling cerebral ischemia

When analyzing the level of S100b and NSE in the animals of group "Glib + iFDE-5 + CI" and "Glib + EPO + CI" was observed by increasing the concentration of markers of damage. Their concentration is statistically significantly different from concentration intimnyh rats ($p < 0.05$) (figure 15).

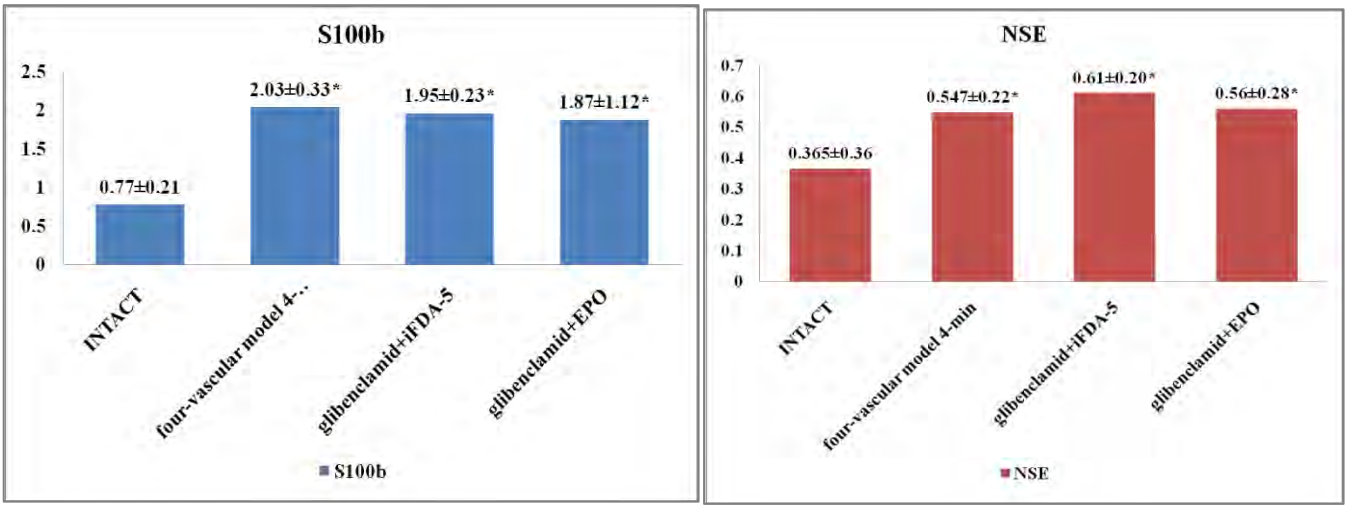

Fig. 15. Effect of tadalafil (iPDE-5), "Epocrin" with the prior introduction of glibenclamid on the concentration of markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) (M ± m; n =10).
Note – * $p<0.05$, # – $p < 0.05$ in relation to intact rats

Histological preparations of the brain of rats of the group "Glib + EPO + CI" similar to the preparations of the group "Glib + iFDE-5 + CI". These groups were observed scattered neurons, the neurons hyperchromia to 83.6% and 70% in the frontal lobes, 63.4% and 80% in the area of the hippocampus; hypochromia neurons to 10% and 20% in the frontal lobes, 26.6% and 10% in the area of the hippocampus; neurons with two ravines neurons in the frontal lobes absent, in the area of the hippocampus, their number corresponded to 10% and 10%. Also recorded pericellular and perivascular edema, hyperemia of the capillaries.

Based on the measurements obtained after morphometric studies and examination of brain slices, it is clear that glibenclamid preconditioners cancels the action of tadalafil and recombinant erythropoietin (figures 16, 17).

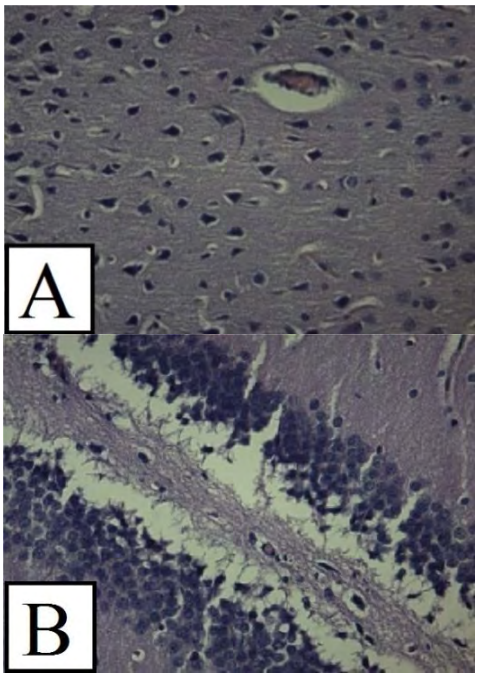

Fig. 16. Brain Slices of the rat group "Glib +iFDE-5+ CI":
A – frontal lobe, X 400, env. hematoxylin+eosin;
B – hippocampus, X 400, env. hematoxylin+eosin

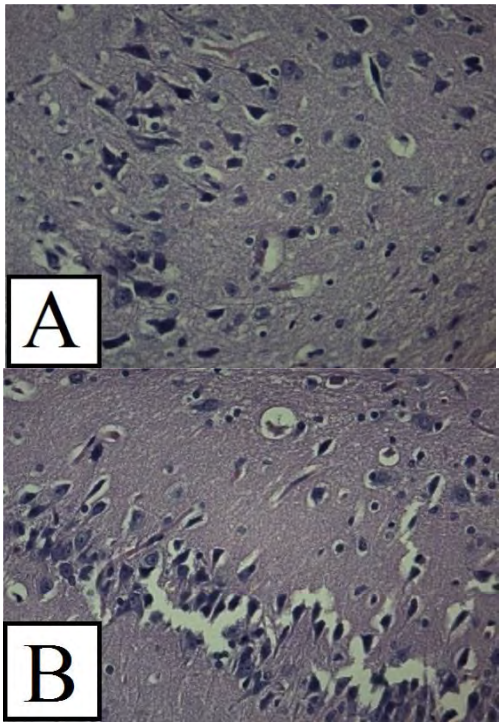

Fig. 17. Brain Slices of the rat group "Glib +EPO+ CI":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus, X 400, hematoxylin+eosin

In the result of the study was chosen as the optimal model to study four-vascular model 4-minute ischemia of the brain with the justification of a temporary simulation mode.

A single administration of tadalafil (1 mg/kg) and "Epocrin" (50 IU/kg) led to rapid recovery of EEG amplitude after the ischemic period, preserving the electrophysiological activity of the retina, improve behavioral status, reducing the level of neurological deficit, markers of brain damage S100b and NSE, to increase the number of hypochromic and presence of two nuclei in neuron in histological sections of brain.

Prophylactic administration of a neuroprotectant "Gliatilin" (85.7 mg/kg) did not produce positive results. The performance of rats in this group did not differ from indicators of control CI ($p > 0.05$).

Concomitant use of prophylactic administration of tadalafil (1 mg/kg) and neuroprotectant "Gliatilin" (85.7 mg/kg) has an additive cerebroprotective effect, which is manifested in the improvement of all criteria for the integrated assessment of this pathology.

Prior administration of glibenclamid (5 mg/kg) neutralized the positive effects tadalafil (1 mg/kg) and "Epocrin" (50 IU/kg) ischemic preconditioning, confirming the implementation of cerebroprotective by preconditioning, with the participation of ATP-sensitive potassium channels.

For visualization of the obtained data was constructed charts on indicators of behavioral status and neurological deficits of the animals (as a percentage) calculated area of each shape, and dynamics of changes in markers of brain damage (figure 18-23). The smaller the area of the figure of the group, the harder the degree of ischemia of the rat brain (figure 23).

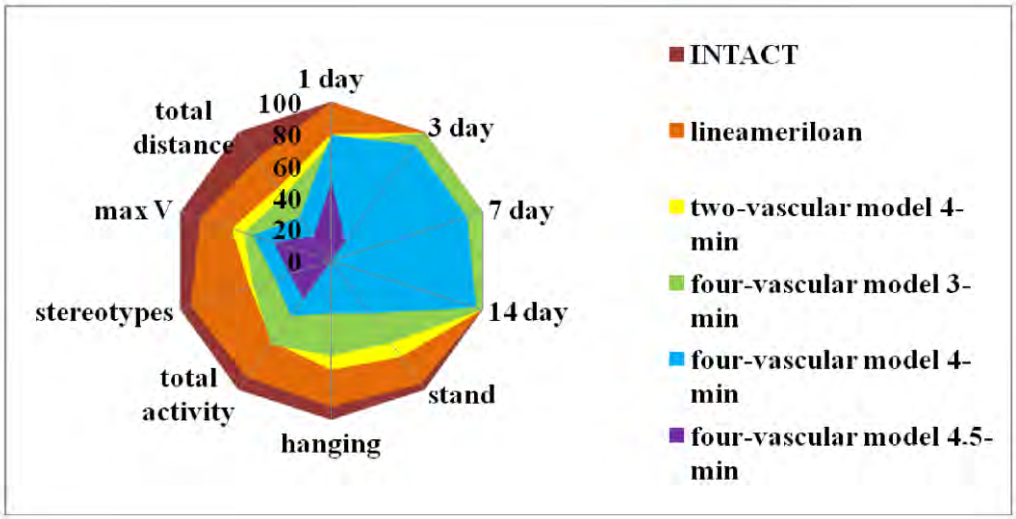

Fig. 18. Impact of duration and severity of the ischemic episode on the indicators of activity of animals in the experiment

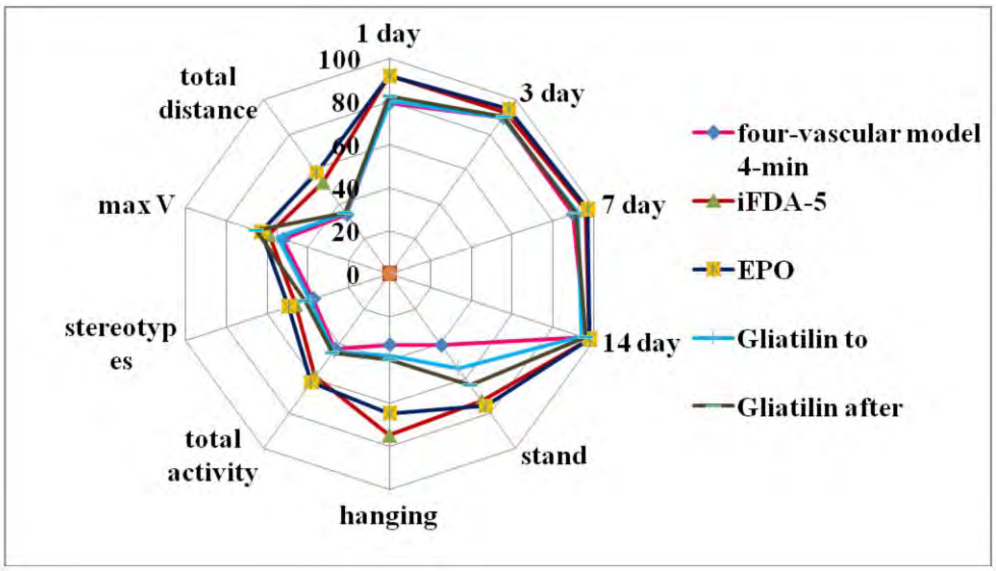

Fig. 19. Influence of used drugs on the activity rate of animals with cerebral ischemia

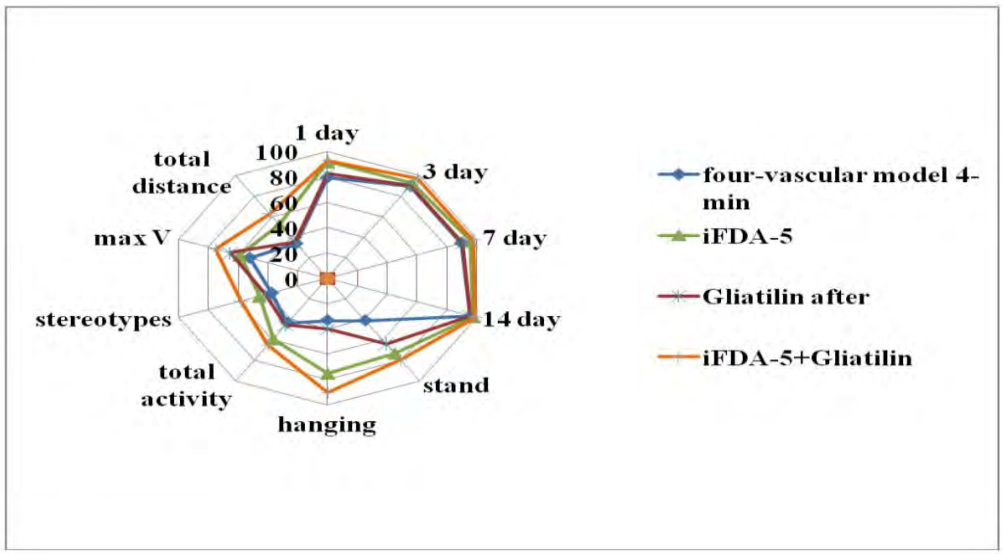

Fig. 20. Influence of used drugs on the activity rate of animals with cerebral ischemia

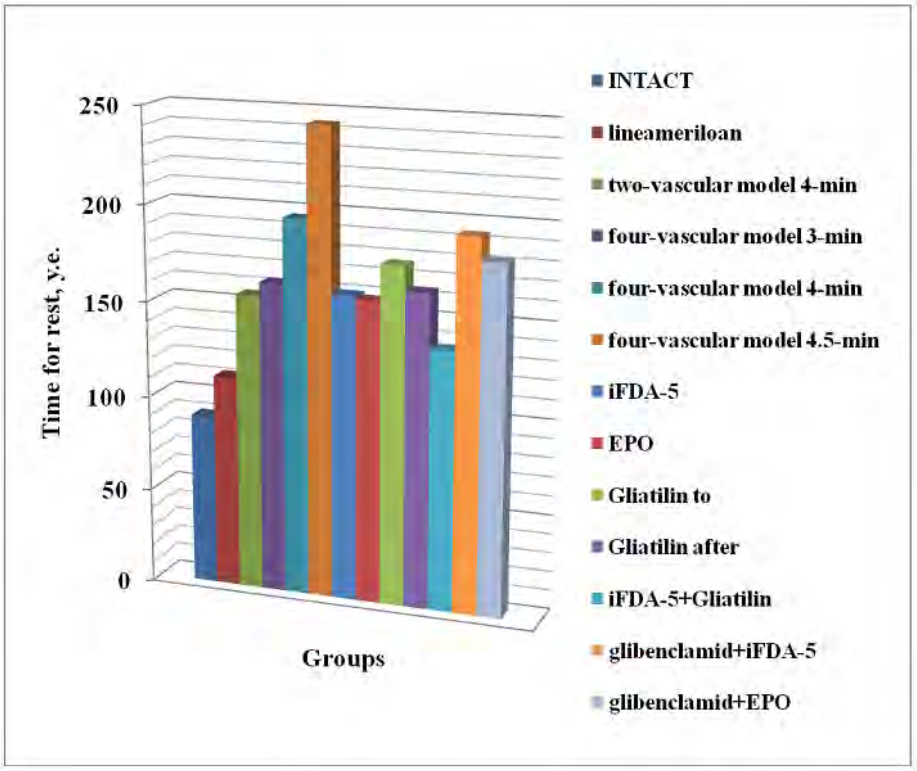

Fig. 21. Influence of used drugs on the duration of stay of animals in the test of actimetry

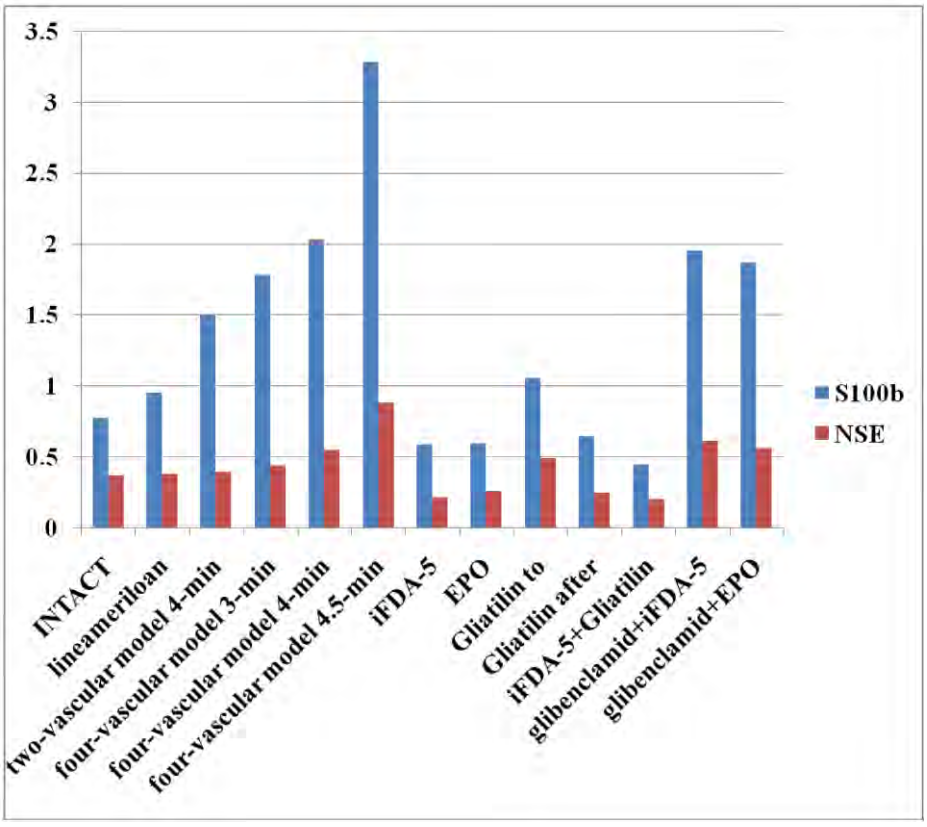

Fig. 22. Influence of used drugs on the level of specific markers of damage of the rat brain S100b and NSE

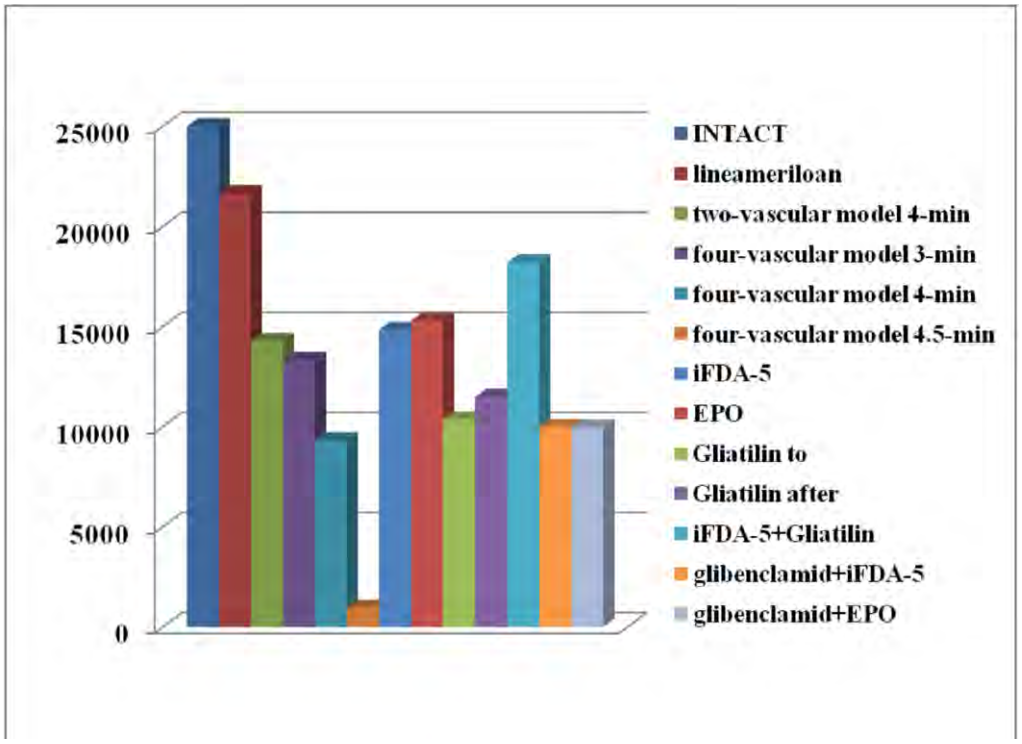

Fig. 23. Area values radar chart of the experimental groups

The received results convincingly testify to the long term development of pharmacological methods and approaches of correction of ischemic brain injury based on triggering mechanism of pharmacological preconditioning. To do this, but recombinant erythropoietin can be used inhibitor of phosphodiesterase type 5.

Conclusions

1. Four-vascular model the 4-minute model of brain pathology in rats was characterized by the development of neurological deficits (of 2.05±0.49 points); a decrease in the number of racks in 2 times and hanging 2.7 times roll "Elevated cross maze"; violation of behavioral status in the test of actimetry, which was manifested in the decline in overall activity in 2 times, reducing the number of patterns of movement in 2 times, reduce the maximum speed by 1.5 times, decrease of passing the total distance of 2.3 times the increase in leisure time is 1.7 times; the increase in the concentration of damage markers S100b 2.5 times and the NSE in 2 times; morphological changes: the presence of 90% hyperchromic neurons in the frontal lobes and the hippocampus.

2. Prophylactic intraperitoneal administration (60 min) of PDE-5 inhibitor, tadalafil (1 mg/kg) exerted cerebroprotective effect in modeling of ischemia-reperfusion, expressed in reducing the severity of neurological deficit (0.8±0.21 points), compared with the control group (of 2.05±0.49 points); increase in the number of stands at 1.7 times and 2.2 hanging times; not a big increase in overall activity, patterns of movement, maximum speed, total distance increased 1.5 times, decrease rest time by 1.2 times; the reduction in the concentration of damage markers S100b 3.5 times and the NSE in 2 times. A number of distinctive characteristics the morphometric study, as well as a set of symptoms, manifestations of behavioral reactions confirm the fact of cerebroprotective properties of tadalafil in comparison with the control group animals.

3. Prophylactic intraperitoneal administration of recombinant erythropoietin «Epocrin» (50 IU/kg) exerted cerebroprotective effect in modeling of ischemia-reperfusion, expressed in reducing the severity of neurological deficit (0.8±0.21 points) compared with the control group (of 2.05±0.49 points); increase in the number of racks and hanging 2 times; not a big increase in overall activity, patterns of movement, maximum velocity, an increase in the total distance of 1.7 times, reduction of time of stay 1.2 times; the reduction in the concentration of damage markers S100b in 3.3 times and the NSE in 2 times. Morphometry

confirmed the neuroprotection "Epocrin" the brain of the rats.

4. The use of intraperitoneal prophylactic neuroprotectant "Gliatilin" (85.7 mg/kg) had a weak cerebroprotective action, which is expressed in the acceleration of recovery of neurological deficit (2.0±0.46 points); no significant increase in the number of racks and hanging, overall activity, patterns of movement, maximum speed, total distance and decrease rest time; not a significant prevention of the increase of the values of neuron specific enolase and protein S100b in the blood serum. Therapeutic use "Gliatilin" (85.7 mg/kg) exerted a more pronounced cerebroprotective action compared to the prophylactic administration of the drug, which is manifested in the acceleration of recovery of neurological deficit (1.75±0.13 points) and locomotor activity compared with the effects of prophylactic purpose. Morphometry in the application of "Gliatilin" as in prophylactic and therapeutic purposes show a lower neuroprotective response in comparison with tadalafil.

5. Concomitant use of prophylactic administration of tadalafil (1 mg/kg) and neuroprotectant "Gliatilin" (85.7 mg/kg) has an additive cerebroprotective action, which is expressed in the presence of mild neurological deficit (0.55±0.07 points); increase in the number of racks in 2 times and hanging 2.6 times; an increase in overall activity and patterns of movement by 1.5 times, the maximum speed of 1.4 times, an increase in the total distance in 2 times, decrease rest time to 1.5 times; approximation of values of neuron specific enolase and protein S100b in the blood serum to indicators of intact animals. Morphometry of combined use of tadalafil (1 mg/kg) and neuroprotectant "Gliatilin" (85.7 mg/kg) significantly increase the resistance of rat brain to ischemia-reperfusion injury compared with the monotherapy.

6. Blockade of K+ATP- channels glibenclamid (5 mg/kg) removes the effects of pharmacological preconditioning of the PDE-5 inhibitor and tadalafil does not affect neuroprotection mediated "Gliatilin".

Conflicts of interest

The authors have no conflict of interest to declare.

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