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For correspondence: Zaporizhia State Medical University, Maiakovskoho avenue 26, Zaporizhia, Ukraine, 69035

E-mail: aupolkovnikov@gmail.com

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ORCID IDs
Oleksii Polkovnikov: https://orcid.org/ 0000-0003-2174-9849
Sergii Pavlov: https://orcid.org/ 0000-0003-3924-4515
Igor Belenichev: https://orcid.org/ 0000-0003-1273-5314
Nataliya Matolinets: https://orcid.org/ 0000-0001-6656-3621

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Author Contributions
Conceptualization: Alexey Polkovnikov;
Results of study: Alexey Polkovnikov, Sergii Pavlov, Igor Belenichev, Nataliya Matolinets;
Writing: Alexey Polkovnikov, Sergii Pavlov, Igor Belenichev, Nataliya Matolinets;
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Endothelial dysfunction under experimental subarachnoid hemorrhage. Possible ways of pharmacocorrection

Oleksii Polkovnikov, Sergii Pavlov, Igor Belenichev, Nataliya Matolinets[1]

Zaporizhia State Medical University, Zaporizhia, Ukraine
[1]Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Aim of the article. To study the effect of Acelysine and Nimodipine on certain endothelial dysfunction indicators and to evaluate their therapeutic efficacy after subarachnoid hemorrhage in rats.

Materials and Methods. An experimental study was carried out using 50 Wistar rats of both sexes. Spontaneous subarachnoid hemorrhage was simulated in animals. Three groups of animals were identified: a control group, a group of animals that received a standard therapeutic dose of Acelysine, and a group that received a standard therapeutic dose of Nimotop. Each group included 15 animals. Also, there were 5 intact animals. Animals were withdrawn from the experiment on days 4 and 7 after determining their motor and exploratory activity. Motor and exploratory activity were determined following SAH with the "Open Field" technique. Biochemical markers of endothelial dysfunction were performed in the rat's brain homogenate.

Results and discussion. The simulation of subarachnoid hemorrhage (SAH) was found to lead to oxidative stress development and the production of increased oxidative modification of proteins (nitrotyrosine (Ntz) on the 4th and especially on the 7th day of the experiment) in brain tissues.

Starting from the 4th day, we registered a compensatory increase in the activity of NO-synthase (NOS) – by 56%, followed by a decrease in its activity on the 7th day, by more than 33% as compared to the intact group of animals. A compensatory increase in VEGF-A in rats with SAH simulation was registered on the 4th day of the experiment, which was further decreased on the 7th day. The established pathobiochemical changes in the brain tissue were accompanied by the development of cognitive deficit in experimental animals, especially on the 7th day of SAH. SAH led to a significant decrease in the total activity of animals by 2.63 times, a decrease in the distance traveled by animals by 1.89 times, the number of freezes increased by 1.86 times, and the immobility of animals increased when moving from the periphery to the center and immobility in the center of the arena (anxiety, fear, disorientation), as well as a decrease in the distance traveled and the speed of movement in the illuminated center of the arena by 2 and 2.6 times, respectively.

Experimental therapy with Acelysine 15 mg/kg led to the normalization of biochemical indicators of endothelial dysfunction: concentration of nitrotyrosine, starting from the 4th day of the experiment, increased eNOS activity and VEGF-A concentration (by 75% and 64% on 7th day).

The administration of Namidopine led to less pronounced effects, statistically significant changes occurred only in relation to VEGF-A concentration. Administration of Namidopine resulted in only a slight increase in VEGF-A concentration.

Acelysine and Nimotop significantly increased the total activity of rats on the 7th day after SAH by 76.3% and 48.8%, respectively. In animals treated with Acelysine, anxiety and fear decreased. Animals were less aggressive and more empathic – long-term grooming increased by 3 times. The administration of Nimotop in rats that survived SAH had a less pronounced positive effect on behavior. Nimotop did not affect indicators of general activity and did not increase the total distance traveled. Animals that received Nimotop were inactive by the 7th day of treatment.

Conclusions. Experimental therapy with Acelysine led to the normalization of biochemical parameters of endothelial dysfunction, namely nitrotyrosine concentration, starting from the 4th day of the experiment and increased eNOS activity and VEGF-A concentration. It should be noted that, in contrast to rats in the control group, under the prescription of Acelysine, we observed an increase in the concentration of eNOS and VEGF-A both on the 4th and 7th days of the experiment. The administration of Namidopine led to less pronounced effects, statistically significant changes occurred only in relation to VEGF-A concentration.

The administration of Acelysine to animals after SAH had a beneficial effect on the emotional status and behavior of animals and also led to the normalization of their general activity and orientation-exploratory activity. The mechanism of the endotheliotropic effect of Acelysine, in our opinion, is associated with its antioxidant effects, modulating impact on endothelial NOS, as well as its property, indirectly, to influence the increased VEGF content. Nimotop therapy had no effect on the emotional status and behavior of the animals. The use of calcium channel blockers revealed such side effects as depression, drowsiness, diplopia, and disorientation.

Keywords: subarachnoid hemorrhage, endothelial dysfunction, acelysine, Nimotop.

Ендотеліальна дисфункція в умовах експериментального субарахноїдального крововиливу. Можливі шляхи фармакокорекції

Олексій Полковніков[1], Сергій Павлов[1], Ігор Беленічев[1], Наталія Матолінець[2]

[1] *Запорізький державний медичний університет, м. Запоріжжя, Україна*
[2] *Львівський національний медичний університет імені Данила Галицького, м. Львів, Україна*

Мета роботи: дослідження впливу ацелізіну та німодипіну на окремі показники ендотеліальної дисфункції та оцінка їх терапевтичної ефективності в умовах експериментального субарахноїдального крововиливу у щурів.

Матеріали та методи: Експериментальне дослідження виконано з використанням 50 щурів лінії Вістар обох статей. У животных моделировалось спонтанное субарахноидальное кровоизлияние. Було виділено 3 групи тварин: контрольна група, група тварин які одержували ацелізин в стандартній терапе-

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Е-пошта: aupolkovnikov@gmail.com

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ORCID IDs
Олексій Полковніков: https://orcid.org/0000-0003-2174-9849
Сергій Павлов: https://orcid.org/0000-0003-3924-4515
Ігор Беленічев: https://orcid.org/0000-0003-1273-5314
Наталія Матолінець: https://orcid.org/0000-0001-6656-3621

Конфлікт інтересів: автори декларують відсутність конфлікту інтересів.

Особистий внесок авторів
Концепція: Олексій Полковніков;
Результати досліджень: Олексій Полковніков, Сергій Павлов,

Ігор Беленічев, Наталія Матолінець; *Написання статті:* Олексій Полковніков, Сергій Павлов, Ігор Беленічев, Наталія Матолінець;

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Дозвіл комісії з біоетики щодо проведення досліджень: Протокол засідання комісії з біоетики Запорізького державного медичного університету № 4 від 24.05.2018р.

Фінансування: часткове фінансування Запорізьким державним медичним університетом у рамках кафедральної наукової тематики «Оптимізація діагностики та хірургічних методів корекції порушень мозкового кровообігу», державний реєстраційний номер: 0118U004255.

втичній дозі і група яка отримувала німодипин в стандартній терапевтичній дозі. Кожна група включала в себе 15 тварин. Також було 5 інтактних тварин. Виведення тварин з експерименту було на 4 і 7 у добу після визначення рухової та пошукової активності. Визначення рухової і пошукової активності проводилося після САК за допомогою методики «Відкрите поле». Визначення біохімічних маркерів ендотеліальної дисфункції виконували у гомогенаті головного мозку щурів.

Результати дослідження та їх обговорення. Було встановлено, що моделювання субарахноїдального крововиливу (САК) призводило до розвитку оксиадтивного стресу та збільшення вмісту у тканинах головного мозку продукту окисної модифікації білків – нітротирозину (Ntz) на 4 та особливо, на 7 добу. Починаючи з 4 доби, було зареєстроване компенсаторне підвищення активності NO-синтази (NOS) на 56 %, з послідуючим зниженням її активності на 7 добу, більш ніж на 33 % по відношенню до інтактної групи тварин. Також було зареєстровано компенсаторне підвищення VEGF-A у щурів з моделюванням САК на 4 добу та подальше його зниження на 7 добу. Встановлені патобіохімічні зміни мозкової тканини супроводжувались розвитком когнітивного дефіциту у дослідних тварин, особливо на 7 добу САК. САК призводило до достовірного зниження загальної активності тварин в 2,63 рази, зниження пройденої відстані тваринами в 1,89 раз, кількість замираний збільшилася в 1,86 раз і збільшувало нерухомість тварин при переході з периферії в центр і нерухомість в центрі арени (тривога, страх, дезорієнтація), а також зниження пройденої відстані і швидкості пересування в освітленому центрі арени в 2 і 2,6 разів відповідно.

Експериментальна терапія ацелізином 15 мг/кг призводила до нормалізації біохімічних показників ендотеліальної дисфункції, а саме, вмісту нітротирозину, починаючи з 4 доби експерименту; підвищення активності eNOS та вмісту VEGF-A (відповідно на 75% та 64% на 7 добу). На тлі призначення ацелізину, відбувалось підвищення концентрації eNOS та VEGF-A як на 4, так і на 7 добу експерименту. Призначення намидопину призводило лише до незначного підвищення вмісту VEGF-A.

Ацелізин та німотоп достовірно збільшували загальну активність щурів на 7-у добу після САК на 76,3% і на 48,8% відповідно. У тварин, які отримували ацелізин, знижувалася тривожність і страх. Тварини були менш агресивні і більш емпатічним - у них в 3 рази збільшилася кількість тривалого груммінга. Введення щурам з САК німотопу надавало менш виражену позитивну дію на поведінку. Німотоп не впливав на показники загальної активності і не збільшував загальну пройдену відстань. Тварини, які отримували німтоп до 7-ї доби лікування були малоактивними.

Висновки. Експериментальна терапія ацелізином призводила до нормалізації біохімічних показників ендотеліальної дисфункції, а саме, вмісту нітротирозину, починаючи з 4 доби експерименту; підвищення активності eNOS та вмісту VEGF-A. Варто зазначити, що на відміну від щурів контрольної групи, на тлі призначення ацелізину, відбувалось підвищення концентрації eNOS та VEGF-A як на 4, так і на 7 добу експерименту. Призначення німотопу призводило до менш виражених ефектів, статистично вірогідні зміни відбувались лише по відношенню до вмісту VEGF-A.

Введення тваринам після САК ацелізину надавало сприятливу дію відносно емоційного статусу і поведінки тварин, а також призводило до нормалізації загальної активності і орієнтовно-дослідницької активності тварин. Механізм едотеліотропної дії ацелізину, на нашу думку, пов›язаний з його антиоксидантними ефектами, модулюючім впливом на ендотеліальну NOS, а також його здатністю, опосередковано, впливати на підвищення вмісту VEGF. Терапія німотопом не мала ефекту щодо емоційного статусу і поведінки тварин. Крім того, при застосуванні блокаторів кальцієвих каналів виявлені побічні ефекти у вигляді депресії, сонливість, диплопія, порушення орієнтації.

Ключові слова: субарахноїдальний крововилив, ендотеліальна дисфункція, ацелізин, німотоп.

Introduction

Subarachnoid Hemorrhage is a severe subtype of stroke that affects patients at an average age of 55 years and results in years of productive life lost. In 85% of cases, hemorrhage is caused by a ruptured cerebral aneurysm [1]. The frequency of subarachnoid hemorrhage in population studies, including out-of-hospital lethal cases, is 9.1 cases per one hundred thousand people per year with some regional variations [2]. Despite the development of technologies for neuroimaging and occlusion of cerebral aneurysms and intensive care methods improvement, 30-day and pre-hospital mortality, it is, unfortunately, still high – about 35% and 15%, respectively [3].

Complications of subarachnoid hemorrhage determine the severity of the disease and negatively affect both survival and functional outcome. They are classically described by the time of their development after the rupture of the aneurysm. There are complications of the acute phase – early brain injury (EBI) (first 72 hours), complications of the subacute phase (3-30 days) and late phase (after 30 days) [4].

Complications in patients with ruptures of intracranial arterial aneurysms are most often: angiospasm (37.2%); intracerebral and intraventricular hemorrhages (27.6%); delayed cerebral ischemia (22.2%); intraoperative complications (9.1%); re-rupture of the aneurysm (6.5%). Probable criteria for predicting the adverse course of the postoperative period are surgery to exclude the aneurysm in patients with a high degree on the Hunt-Hess scale (> 2.7 ± 0.2) and the WFNS scale (> 2.1 ± 0.2). In the acute period (1-14 days), postoperative mortality in transcranial interventions (16.2%) differs compared with the group of embolization (10.2%) [5].

A ruptured aneurysm triggers pathophysiological processes that lead to brain damage and neuronal death. A sudden increase in intracranial pressure due to extravasation of blood into the subarachnoid space, as well as acute vasoconstriction, provoke a decrease in cerebral perfusion pressure (CPP), autoregulation impairment, and in severe cases, transient or persistent ischemia. The blockade of the subarachnoid cerebrospinal fluid spaces after *hemorrhage, and in severe cases intraventricular hemorrhage, leads to the development of acute occlusive hydrocephalus, increasing intracranial hypertension.* Neuron death and endothelial injury can lead to cytotoxic edema and blood-brain barrier destruction resulting in vasogenic edema development. In addition, failure of microcirculation, microthrombosis, changes in ionic homeostasis, excitotoxicity, oxidative stress and neuronal swelling lead to cell death [6,7]. The clinical picture reflects all of the above and determines both the initial severity of the patient's condition and the result of treatment.

The pathogenesis of delayed cerebral ischemia has not yet been sufficiently studied. A recent literature review shows that for 3 to 14 days following an aneurysm rupture, 70% of patients may develop vasospasm. However, only 30% of them develop delayed cerebral ischemia (DCI). The latter is also observed in 21% of patients who don't have vasospasm which can be diagnosed angiographically [7,8,9].

Recent studies show that brain endothelial cells have additional functions compared to the endothelium of the peripheral vascular network, such as facilitating information transfer between neurons and glial cells and blood-brain barrier (BBB) support [10,11]. Also, endothelial cells control the vascular tone and blood circulation due to the balance of secretion between vasoconstrictors (endothelin-1 (ET-1) and thromboxane (TxA2)), as well as vasodilators (nitrogen oxide (NO), prostacyclin (PGI2)), endothelium hyperpolarizing factor (EDHF) [12].

Patients who survived DCI after aneurysmal SAH, have an increased risk of unfavorable outcomes. It is assumed that vasospasm is the main culprit of DCI, but recent studies show that several pathogenesis models are involved. Endothelial cells involved in all stages of ischemic cascade following rupture of an aneurysm are therapeutic targets. Stabilization of endothelial cell function in the acute phase may reduce the symptoms of cerebral edema by minimizing blood-brain barrier dysfunction. Delayed-phase of protection can reduce endothelial cell apoptosis, microthrombosis and cerebral vasospasm. The mechanisms of specific effects on endothelial cells, as well as the results of these effects, are still poorly under-

stood. However, they could be considered as a central mediator of both early and delayed brain damage [13].

The above is pathogenetic substantiation of an effective pharmacocorrection of subarachnoid hemorrhage outcomes with medicines of endothelioprotective properties. Current experimental clinical researches show the effect of Acelysine (soluble form of acetylsalicylic acid in combination with L-lysine) and Nimodipine (calcium channel blocker) on certain endothelial dysfunction indicators. In hospitals, these medications are used as components of antiplatelet therapy and for the prevention of angiospasm. However, the mechanism of their endothelioprotective action after subarachnoid hemorrhage has not been established, which determines the relevance of further research in this direction. This aims were to study the effect of Acelysine and Nimodipine on certain endothelial dysfunction indicators and to evaluate their therapeutic efficacy after subarachnoid hemorrhage in rats.

Materials and Methods: an experimental study was carried out using 50 Wistar rats of both sexes weighing 170-230 g (10-12 weeks before the start of the experiment), which have undergone preliminary acclimatization for 14 days [14].

Rats are obtained from the nursery of the Institute of Pharmacology and Toxicology of the Academy of Medical Sciences of Ukraine. Care, maintenance and feeding of animals will be carried out under standard conditions of a stable microclimate and during 12-hour daylight hours in the Vivarium of the Department of Experimental Research of Zaporizhia State Medical University (ZSMU) of the Ministry of Health of Ukraine. Laboratory animals' diet is based on the standardized feed "Rezon-1" Passage trap-120-1 with free access to food and water, stress factors are excluded. Rats are kept in standard conventional polycarbonate cages (Tecniplast S.p.A., Italy) with dimensions of 610x435x-215mm or 335x235x190mm for 5 animals.

Three groups of animals were identified: a control group, a group of animals that received a standard therapeutic dose of Acelysine, and a group that received a standard therapeutic dose of Nimodipine. Each group included 15 animals. There were also 5 intact animals. Only healthy animals were included in the study. The animals were randomly assigned [15].

Animals were marked with 1% Brilliant Green solution. Before the start of the study, the ZSMU Bioethics Commission checked and approved the research plan, as well as all procedures related to animal keeping and humane treatment and their use in the experiment (minutes of the meeting of the Commission on Bioethics of Zaporizhia State Medical University No. 4 dated May 24, 2018). The study was carried out in compliance with Directive 2010/63EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, as well as with the national "Ethical Principles for Scientific Experiments on Animals" (Ukraine, 2001) and the guidelines set out in "Basic principles of studying the toxicity of potential drugs" (State Pharmacological Center of Ukraine, Kyiv, 2000).

Before the experiment, for the purpose of domestication, rats have been held in hands for 2-3 minutes within 5 days, which facilitated the following experimental studies.

Study design: Anesthesia was induced with intravenously administered sodium thiopental 40 mg/kg into the tail vein with a 26G needle. Before anesthesia using sodium thiopental, rats have been warmed up under a heat lamp for 5 minutes to dilate the tail vein. The surgery was performed under aseptic conditions on a heated table (37°C) to maintain body temperature during the procedure.

For disinfection and occipital puncture, certain areas were wiped with chlorhexidine in 70% ethanol. Experimental animals underwent suboccipital puncture under general anesthesia (sodium thiopental 30 mg/kg). The SAH model was created based on R.V. Dudhani et al method [16], but autologous blood was administered only once, and aspiration of cisternal CSF was not performed. 0.20 ml of blood from the tail vein was taken by a syringe with heparin.

A puncture of the large occipital cistern of the subarachnoid space was performed, after which 0.20 ml of previously collected *autologous blood* was injected. SAH as a manifestation of hemorrhagic stroke develops immediately

after injection of venous autologous blood into the subarachnoid space. After surgery, 6% glucose solution is fed into a drinking bowl next to a bottle of water, allowing animals to drink one or the other. The food is also placed at the bottom of the cage to facilitate eating.

Nimodipine was injected intraperitoneally with a dosage of 0.3 mg/kg once every 8 hours (8 AM, 4 PM, 12 AM), Acelysine – 15 mg/kg once a day at 10 AM.

Animals were withdrawn from the experiment on days 4 and 7 after the motor and exploratory activity had been determined. Motor and exploratory activity were determined following VH with the "Open Field" technique using an arena of our own production with dimensions of 80x80x35cm. The animal was placed inside one of the sides with its muzzle to the wall, then it was allowed to move freely around the arena for 8 minutes. We assessed the total distance traveled (cm), general motor activity (cm2/s), activity structure (high activity, low activity, inactivity, %), the number of fading and entering the center, the distance traveled near the wall (cm) and in the center of the arena (cm, %), vertical search activity (the number of stands on hind legs near the wall and in the center), the number of short and long grooming events, the number of defecation and urination acts. In addition, the Rotarod test was performed to assess coordination and endurance [17]. The studies were carried out based on the Department of Experimental Pathophysiology and Functional Morphology of the Educational Medical Laboratory Center of Zaporizhia State Medical University. Experiments were conducted in a well-lit room in complete silence. During experiments, the influence of external and internal visual, olfactory and auditory stimuli was excluded. Animals' *behavioral assessment* was done by a laboratory assistant who didn't know if animals belonged to a particular experimental group. Image capture and recording were carried out using an SSC-DC378P color video camera (Sony, Japan). The video file was analyzed using the Smartv 3.0 software (HarvardApparatus, USA).

Biochemical markers of endothelial dysfunction were determined in a rat brain homogenate with experimental simulation of subarachnoid hemorrhage on days 4 and 7. The tissue was homogenized in buffer (50 mM Tris-HCl, 5 mM EDTA, 1 mM DTT, 1% Triton X-100), pH 7.5 at 40C in a ratio of 1:6 tissue/buffer and centrifuged at 13000g. Markers were detected by the enzyme-linked immunosorbent assay based on the use of a "sandwich" variant of a solid-phase enzyme-linked immunosorbent assay. The procedure was performed with immune complex ImmunoChem-2100 (USA). The assay was done in 96-well microplates, the bottom of the wells was coated with monoclonal antibodies to the corresponding molecular marker [18]. Experimental samples were tested for Nitrotyrosine (Ntz), Elabscience, Catalog No: E-EL-0040, ng/mL; Rat VEGF-A (Vascular Endothelial Cell Growth Factor A), Elabscience, ELISA Kit, pg/mL and Rat NOS3/eNOS (Nitric Oxide Synthase 3, Endothelial), Elabscience, ELISA Kit, pg/mL.

Statistical processing of results was performed using Microsoft Excel 2016 with the AtteStat 12 statistical processing package. To assess the significance of differences *the Dunn post hoc* method following a significant Kruskal-Wallis test was used in the groups studied. Differences were considered probable at $p < 0.05$.

Results

Experimental studies have shown that the simulation of subarachnoid hemorrhage (SAH) led to the oxidative stress development and the product of oxidative modification of proteins (nitrotyrosine (Ntz) on the 4th and especially on the 7th day of the experiment) increase in brain tissues (Table 1). It is known that nitrotyrosine is a cyto- and genome oxidizing compound and plays a key role in the molecular mechanisms of cell death. Under conditions of oxidative stress, which develops in brain tissues after SAH, oxidative modification of functionally active protein molecules, including antioxidant enzymes, occurs. It leads to depletion of the *intracellular antioxidant pool*, disruption of cellular compensatory capabilities, intensification of free radical oxidation and breakdown of nitrocellulose stress with parallel peroxynitrite accumulation. Peroxynitrite inhibits the activity of thiol compounds and enzymes of the thiol-disulfide system, which leads to a homocysteine pool increase.

Such pathobiochemical processes lead to a decrease in NO bioavailability (hyper-concentration of peroxynitrite and nitrotyrosine) and, as a consequence, disruption of endothelial NO synthase activity [19]. Starting from the 4th day of SH, we registered a compensatory increase in the activity of NO-synthase (NOS) – by 56%, followed by a decrease in its activity on the 7th day, by more than 33% against the intact group of animals (Table 1). The above processes may be possible mechanisms for endothelial dysfunction development following SAH under oxidative stress. In addition, the products of free radical oxidation, including nitrotyrosine, are pro-inflammatory mediators. The compensatory response to the endothelial dysfunction development and associated pathobiochemical processes in cells is migration and maturating of endothelial cells, vascular remodeling. One of the key molecules of angiogenesis and endothelial survival is endothelial growth factor VEGF-A, which is a specific mitogen of endothelial cells and a factor of vascular permeability [20]. In addition, VEGF-A is also a factor of NO-synthase mediated vasodilation and activates monocyte migration. During our studies, a compensatory increase in VEGF-A in rats with SH modeling on the 4th day of the experiment was registered, and its further decrease on the 7th day (Table 1). We consider it a disruption of the compensatory-adaptive capabilities of the organism under the depletion of endogenous antioxidants and an increase in cytotoxic derivative reactive oxygen species, including nitrogen oxide. The established pathobiochemical changes in the brain tissue were accompanied by the cognitive deficit development in experimental animals, especially on the 7th day of the SAH. Thus, when assessing specific indicators of the "open field" technique, the simulation of SAH was found to have an adverse effect on the behavioral characteristics of animals. SAH led to a significant decrease in the total activity of animals by 2.63 times, and a decrease in the distance traveled by animals by 1.89 times. Animals were slow, inactive, anxiously aggressive, disoriented. 1.86 times higher number of freezes was recorded in animals with SAH. The immobility of animals increased when moving from the periphery to the center and immobility in the center of the arena (anxiety, fear, disorientation), as well as a decrease in the distance traveled and the speed of movement in the illuminated center of the arena, increased by 2 and 2.6 times, respectively (Table 2). A decrease in high activity and low activity was also recorded, which also indicated the suppression of the exploratory function of the central nervous system in rats, as well as anxiety and excitability formation in animals after SAH simulation. SAH simulation did not affect the number of free stands of animals but led to an increase in stands against the wall. The number of short grooming acts also decreased followed by an unchanged amount of long-term grooming. This fact also testifies to increased anxiety and excitability of animals and a decreased feeling of comfort which leads to depression. A decrease in high and low activity can also be regarded as a decrease in cognitive abilities since the rat did not make movements aimed to assimilate with a new environment. Our data correspond to the concept of cognitive dysfunction following an acute cerebrovascular accident, TBI. Intracranial hemorrhage leads to persistent cognitive deficits, as well as psycho-emotional disorders – lethargy, fear, anxiety, disorientation, aggressiveness, and irritability [21]. Glutamate excitotoxicity, oxidative

Table 1

Biochemical markers of endothelial dysfunction in rat brain tissues with the simulation of subarachnoid hemorrhage on the 4th and 7th day of the experiment

Groups of animals (n=10)	Nitrotyrosine, ng/ml	eNOS, pg/ml	VEGF-A, pg/ml
Intact	1,7±0,17	25,7±0,6	23,2±0,41
SH, 4th day	6,8±0,41	58,4±1,1	59,4±1,2
SH, 7th day	6,7±0,33	17,1±0,9	28,4±0,75
SH+ Acelysine 15 mg/kg, 4th day	3,2±0,28*	75,2±2,1*	64,2±1,4*
SH+ Acelysine 15 mg/kg, 4th day	4,1±0,17*	68,6±1,7*	78,6±2,1*
SH+ Nimotop 0,3 mg/kg, 4th day	5,9±0,26	60,3±1,1	62,4±1,1
SH+ Nimotop 0,3 mg/kg, 4th day	6,8±0,41	19,3±0,86	28,4±0,75

Note * - p≤0,05 referring to SH

stress, neuroapoptosis, and energy deficiency are considered the main causes of cognitive functions impairment under SAH [22].

Experimental therapy with acelysine 15 mg/ kg led to the normalization of biochemical indicators of endothelial dysfunction: concentration of nitrotyrosine, starting from the 4th day of the experiment, increased eNOS activity and VEGF-A concentration (by 75% and 64% on the 7th day). It should be noted that, in contrast to rats of the control group, under the prescription of Acelysine, there was an increase in the concentration of eNOS and VEGF-A both on the 4th and 7th days of the experiment. The effect of Acelysine on the increase of VEGF-A concentration against the background of eNOS modulation, under the conditions of simulated subarachnoid hemorrhage, which is, in our opinion, the key mechanism of its endotheliotropic effect. Thus, it was shown that under hypoxia and intensification of free radical oxidation, VEGF can bind with tyrosine kinase receptors VEGF and activate them. The binding of VEGF with receptors triggers a signaling cascade that stimulates the growth of vascular endothelial cells and ensures their survival and proliferation. In addition, due to VEGF-mediated stimulation of the permeability of small blood vessels, the entry of plasma proteins through the vessel wall is enhanced and forms an extravascular fibrin gel [9, 10, 19, 20]. This gel is an "ideal" environment for endothelial cell growth, which, under the conditions of simulating subarachnoid hemorrhage, ensures the formation of a capillary network and restricts the development of endothelial dysfunction phenomena [9, 10].

The effects of Nimodipine were less pronounced. Thus, the prescription of Nimotop at a dose of 0.3 mg/ kg every 8 hours did not increase VEGF-A content (Table 1).

Table 2

The effect of Acelysine and Nimotop on indicators of behavior and orientation-exploratory activity of animals in an open field on the 7th day after SAH

Indicator	Intact	VH (control)	Acelysine	Nimotop
Open field				
General activity, cm/s	34175,01±2839,76	14177,12±1188,23[2]	22976,22±3789,11[1]	21156,22±1122,54,[2]
Duration of high activity, %	17,83±1,44	9,72±0,97	14,65±1,22[1]	7,22±2,22[2]
Duration of low activity, %	61,71±7,08	51,21±3,56[2]	55,22±7,34	37,22±5,77[2]
Duration of inactivity center-periphery boundary, %	10,22±2,11	39,67±4,08[2]	14,2±6,17[2,1]	12,2±1,11[2]
Duration of inactivity near the wall, %	30,47±6,59	51,22±8,19[2]	38,2±5,12[1]	89,22±7,26[1,2]
Fading, unit	284±35	767±18[2]	312±21[1,2]	500±28[1,2]
First delay when entering the center, sec	43,21±7,88	199,22±22,8[2]	69,77±5,42[1]	299,8±64,5*
Distance traveled, cm	4161,81±290,78	2887,8±522,2[2]	3278,5±611,5[1]	2278,5±509,22[2]
Average speed of movement in the center without the rest, cm^2/s	77,37±26,31	48,34±4,22[2]	66,8±5,12[2,1]	41,49±4,95[2]
Center distance, cm	122,43±34,61	68,2±5,12[2]	98,31±8,11[1,2]	34,3±3,11[1]
Wall distance, cm	410,44 ±28,55	615,11±900,11[2]	488,11±441,11[1,]2	724,8±188,3[1,2]
Stand near the wall, unit	6±1	1±0[2]	4±0[1,2]	2±0[2]
Free stand, unit	2±1	2±0	2±0	1
Short-term grooming, unit	4±1	1	2	1
Long-term grooming, unit	7±1	1[2]	3[1,2]	1
Defecation, unit	3±1	2	2[1,2]	1[1]
Urination, unit	1	1	1	1

Note: 1 – significant difference (p<0,05) compared with the control group
2 – significant difference (p<0,05) compared with the intact group

Discussion

The endothelioprotective effect of the studied medications was confirmed when researching behavioral reactions in experimental animals. The administration of Nimotop and Acelysine immediately after animals came to from anesthesia had different effects on behavioral responses, emotional status, and cognitive exploratory functions of animals. Thus, Acelysine and Nimodipine significantly increased the total activity of rats on the 7th day after SAH by 76.3% and 48.8%, respectively. Rats that received Acelysine were more mobile and showed greater interest in the environment as compared to the control group of animals. Thus, in this group, the duration of high activity (objects examined above the floor of the arena) and the number of stands near the wall (4 times) significantly increased, which indicated an increase in the active component of exploratory activity associated with an improvement in the cognitive functions of the central nervous system (fig. 1). In animals treated with Acelysine, anxiety and fear decreased, as evidenced by a decrease in inactivity near the wall by 56.3%, a decrease in the time of the first delay when moving to the illuminated center, and a decrease in fading by 26.4% (Fig. 2). Animals that received Acelysine after SAH were well-oriented and moved more freely in the illuminated part of the arena for a day. Their

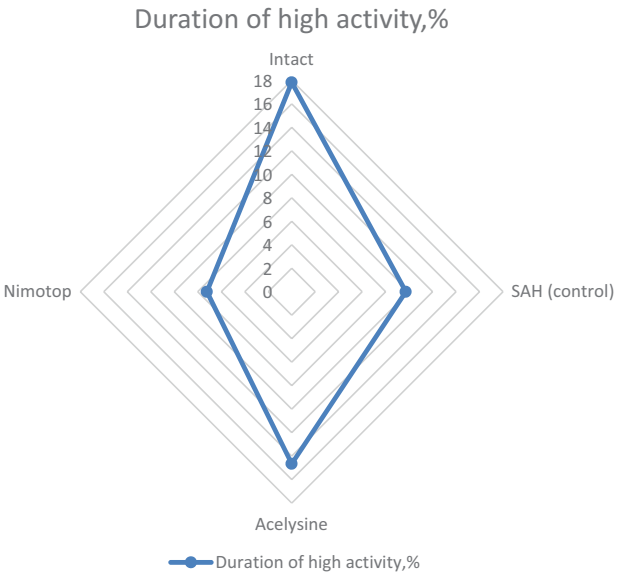

Figure 1. Increase in the duration of high activity (examination of objects above the floor of the arena) in animals receiving Acelysine

speed of movement around the illuminated center increased by 65.5%, and the distance traveled in the illuminated center doubled. The animals were less aggressive and more empathic - long-term grooming increased 3 times (Fig. 3,4). Thus, the administration of Acelysine after SAH had a beneficial effect on the emotional status and behavior of animals in the open field – a decrease in anxiety, aggres-

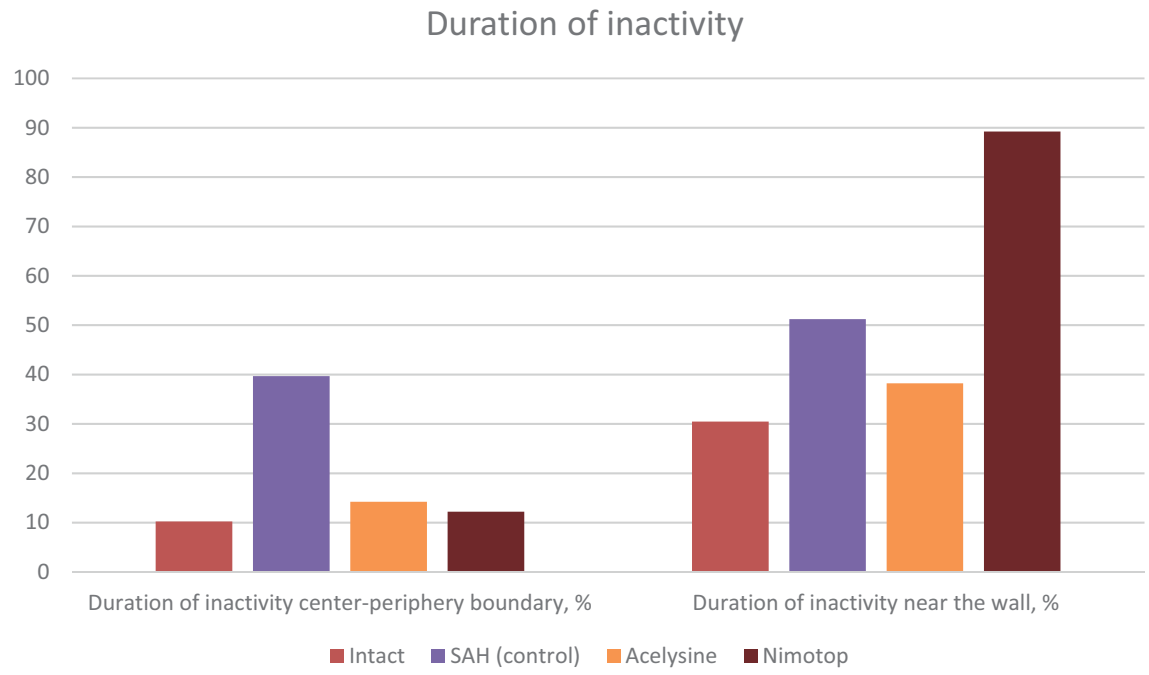

Figure 2. Inactivity against the wall and the time of the first delay in the transition to the illuminated center

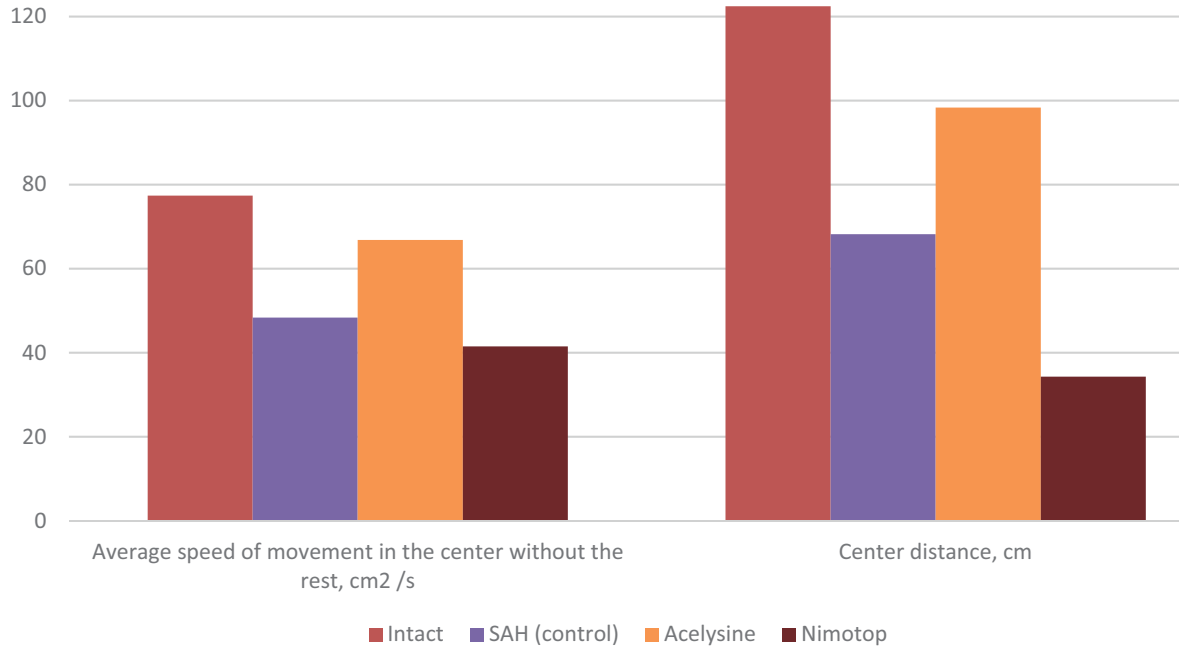

Figure 3. The speed of movement in the illuminated center is 2 times and the distance traveled in the illuminated center

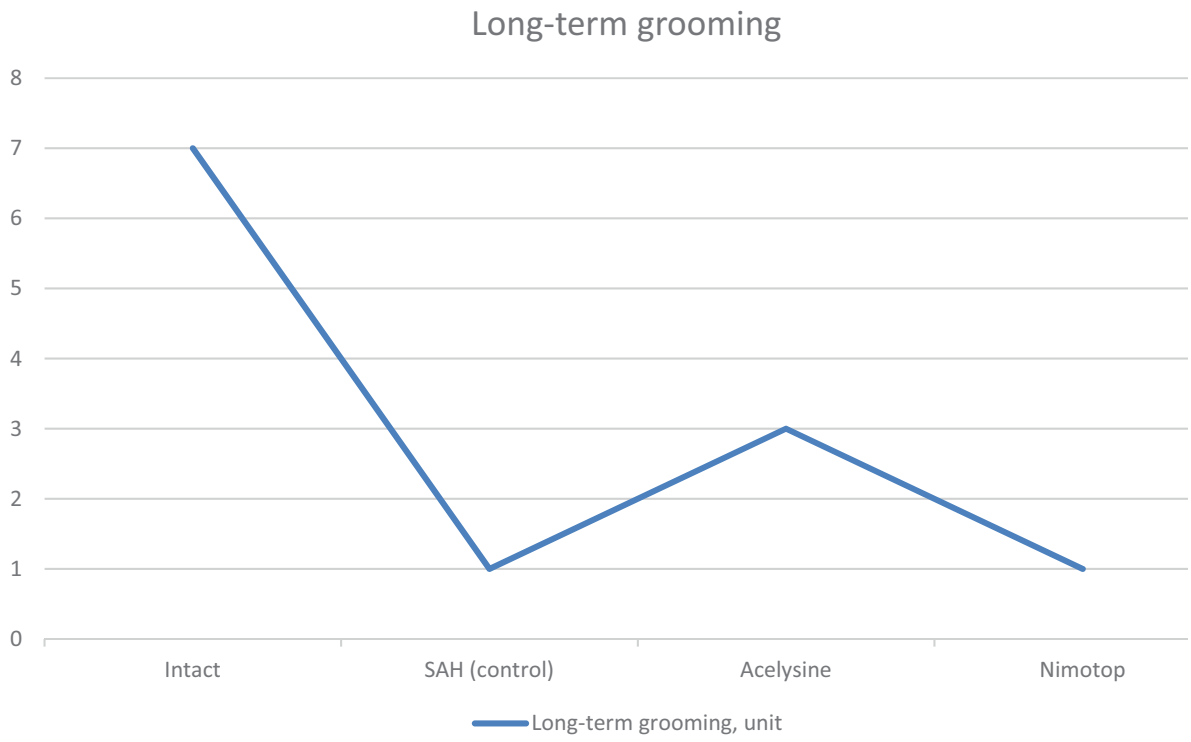

Figure 4. Effect of Acelysine and Nimotop on long-term grooming

siveness, depression and an increase in the comfort and empathy of animals. Acelysine led to the normalization of the general and exploratory activity of animals after SAH (Table 2).

Such effect of Acelysine can be considered from the standpoint of the pharmacology of its active component – L-lysine. L-lysine can convert to pipecolic acid and increase the affinity of GABA receptors, which leads

to a decrease in anxiety and fear and gives the drug an anxiolytic effect. L-lysine reduces NMDA hyperexcitability, decreases excitotoxicity, and preserves the viability of hippocampal and sensorimotor neurons [23]. L-lysine, in combination with acetylsalicylic acid, can provide an endotheliotropic effect – reduce platelet aggregation and increase the expression of eNOS. In addition, antioxidant effects of acetylsalicylic acid associated with its effect on the cyclooxygenase-prostaglandin system have been established [24,25].

The administration of Nimotop in rats that survived SAH had a less pronounced positive effect on behavior. Nimotop did not affect indicators of general activity and did not increase the total distance traveled. Animals that received Nimotop were inactive by the 7th day of treatment. In the Nimotop group, there was no increase in exploratory and orientation activity and no decrease in anxiety. Some indicators of anxiety and low orientational activity were significantly worse than in the untreated animal group: an indicator of high activity, fading, distance in the center. It is possible that Nimotop, as a primary neuroprotector, in the acute period of SAH, realizes its effect exclusively for the survival of neurons, without affecting behavioral and cognitive functions of the central nervous system [19]. In addition, calcium channel blockers were found to have AR (adverse reactions) – depression, drowsiness, diplopia, disorientation. In conclusions: Experimental therapy with Acelysine led to the normalization of biochemical parameters of endothelial dysfunction, namely nitrotyrosine concentration, starting from the 4th day of the experiment and increased eNOS activity and VEGF-A concentration. It should be noted that, in contrast to rats of the control group, under the prescription of Acelysine, an increase in the concentration of eNOS and VEGF-A both on the 4th and 7th days of the experiment was observed. The administration of Nimodipine led to less pronounced effects, statistically significant changes occurred only in relation to the VEGF-A concentration.

The administration of Acelysine to animals after SAH had a beneficial effect on the emotional status and behavior of animals and also led to the normalization of their general activity and orientation-exploratory activity.

The mechanism of the endotheliotropic effect of Acelysine, in our opinion, is associated with its antioxidant effects, modulating impact on endothelial NOS, as well as its property, indirectly, to influence on increase VEGF content.

Nimotop therapy had no effect on the emotional status and behavior of the animals. The use of calcium channel blockers revealed such side effects as depression, drowsiness, diplopia, and disorientation.

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