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Evaluation of the Cerebroprotective Properties of Ademol-gel in the Analysis of Specific Indicators in the Open Field Test

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Experimental subarachnoid hemorrhage (SAH) modeling in rats led to a significant, by 3 times, decrease in the total activity of animals, a decrease in the distance traveled by 2 times, a significant increase in inactivity when moving from the dark field to the illuminated part by 2 times, indicating a decrease in locomotor and search activity. SAH did not affect the number of unsupported rearing, but led to an increase in supported rearing against the wall, and a decrease in the number of short grooming acts against the background of an unchanged amount of long grooming. This behavior also testified to increased anxiety, excitability, irritability of animals, discomfort, and depression. The intranasal introduction of Ademol-gel at a dose of 2 mg/kg (a developed original intranasal form) to animals with SAH contributed to the positive emotional status and behavior of rats in the open field test, as evidenced by a decrease in anxiety, aggressiveness, and depressive behavior against the background of increased comfort and empathy in animals. Against the background of the intranasal introduction of Ademol-gel, the processes of restoring general motor and exploratory activity in animals after SAH were also accelerated. Nimodipine (30 μg/kg) was inferior to Ademol in terms of the degree of influence on the studied parameters of animal behavior. The results are an experimental justification for further study of a new dosage form – Ademol intranasal gel.

Keywords: Ademol, Cognitive Deficit, Subarachnoid Hemorrhage.

According to WHO data, more than 10 million people in worldwide have traumatic brain injury (TBI), of which 250-300 thousand people die. In Europe, TBI is the leading cause of death among people under 35 years of age. Approximately in 10–15 years, the frequency of TBI increases by almost 2 times.[1] TBI has a high and steadily growing prevalence and is more

common in young and middle-aged people (20-50 years) in the most able-bodied population group. Every 10–15 years, the frequency of TBI increases by almost 2 times.[2] According to the literature, the number of deaths after cerebral hemorrhages ranges from 52 to 82 %, and after subarachnoid hemorrhages (SAH) – from 32 to 64%.[2] Stroke is the main cause of disability in the adult population, and only 20% of survivors return to work. Given the above and the variety of clinical manifestations and severity of TBI, it is necessary to develop strategies for evidence-based approaches to the pathogenetic treatment of TBI based on an in-depth study of various aspects of pathogenesis. The urgency of the problem of management of TBI and its consequences is associated with significant economic losses, which are complemented by the persistent disability of 10-12 % of victims.[1] Contemporary medicine has a large range of drugs of pharmacological brain protection for the prevention and treatment of the consequences of TBI, but not all of them meet modern requirements. Therefore, the search and development of new highly effective and safe medicines characterized by polytropic pharmacodynamic effects and aimed at the simultaneous correction of various links in the pathogenesis of TBI, is one of the priority areas for modern fundamental and clinical neurology.

Despite a myriad of drugs affecting cerebral hemodynamics, treating these patients is not effective enough. This is due to the short duration of action of many drugs (Aminophylline, Vinpocetine, and Pentoxifylline), adverse changes in hemodynamics, the development of the intracerebral steal phenomenon. In recent years, drugs from the class of calcium channel blockers, a prominent representative of which is nimodipine, have been used to manage chronic disorders of cerebral circulation.

The main neuroprotective agents include the N- and L-subtype calcium channel blocker Nimotop (3-(2-methoxyethyl) 5-propan-2-yl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate) that prevents the launch of the glutamate-calcium cascade of neuronal damage, exhibits potent membrane stabilizing, fibrinolytic and antioxidant effects. Clinical trials suggest that Nimotop reduces all-cause mortality and neurological deficit in subarachnoid hemorrhage, TBI, and ischemic stroke. [3] Nimodipine has been shown to attenuate early brain damage after TBI with subarachnoid hemorrhage, neurological deficit, and cerebral edema in the experiment.[4]

Among many protective mechanisms of some cerebroprotective drugs used to treat acute cerebral ischemia, particularly in conditions of hemorrhagic stroke (HS), the leading place is occupied by the drug's ability to improve cerebral circulation by reducing reflex vasospasm. The leading place among the many protective mechanisms of action of cerebroprotective agents used to treat acute cerebral ischemia, particularly under conditions of hemorrhagic stroke, is the ability of the drug's ability to improve cerebral circulation by reducing reflex vasospasm. An experimental study of the anti-ischemic properties of a new molecule called Ademol (1-adamantyloxy-3-morpholino-2-propanol hydrochloride) showed that after its parenteral introduction to rats with severe intracerebral hemorrhage (ICH) and SAH, mortality decreased to the level of that after the administration of Mexidol, Citicoline and Nimodipine.[5]

A stimulating effect of Ademol on cerebral circulation in the basin of the internal carotid artery and its modulating action on microcirculation in the cerebral cortex in SAH has been demonstrated as well as its modulating action on central hemodynamics and intracranial pressure, the factors that form stable cerebral blood flow, have been established in various types of acute cerebral ischemia.[6] Recently, intranasal dosage forms of neuroprotectors have been intensively developed as delivery of drugs to the brain is important for the effective prevention and management of brain diseases. The intranasal delivery route has several advantages and may be used to introduce active ingredients manufactured as solutions for injections. The intranasal dosage form provides the highest neuroavailability of the active pharmaceutical ingredient (both due to rapid absorption in the epithelium of the nasal cavity and due to axonal transport) and fast onset of action. After the intranasal introduction, the drug reaches its main site of action in the brain within 5 min, whereas typical oral and intravenous routes have limitations in drug delivery to the brain. Besides, this non-invasive way does not require the participation of medical personnel or special

training. It can be used not only in the acute period of the disease but also later on an outpatient basis, which in turn has certain economic advantages and high compliance with treatment.[7] Therefore, considering all the positive pharmacological effects of adamantane derivatives in TBI and the prospect of nasal drug administration, a nasal form of Ademol was developed and a study of its cerebroprotective properties was conducted on the open field test.

MATERIAL AND METHODS

The method of synthesis of the Ademol substance was developed by the Head of Technology Transfer, Innovation and Intellectual Property Department of Institute of Organic Chemistry NAS of Ukraine Yu.V. Korotkyi. The composition and properties of 1 % Ademol-gel was developed as a fragment of the Research work of the National Pirogov Memorial Medical University, Vinnytsya in collaboration with the Department of Medicines Technology of the ZSMU. Various approaches are commonly used for formulation development: empirical, based on the substantiation of the dosage form components due to the experience of the researcher; the methods of mathematical planning of the experiment, when a single or multifactorial experiment allows to select the components of the formulation by determining the influence of the factor (factors) on the selected optimization parameter; use of software in the form of an expert system that combines in its functionality the possibilities of an empirical approach, methods of mathematical planning of an experiment and built-in machine learning models.[8-10]

In silico research on the choice of the rational composition of the formulation of the nasal form with Ademol was carried out using the ExpSys Nasalia expert system developed at the Departments of Medical and Pharmaceutical Informatics and Advanced Technologies, and Pharmacology and Medical Formulation with Course of Normal Physiology of ZSMU. The system contains machine learning models (blender [random forest, extra tree]) that is a complex model of machine learning, which includes random forest extra tree models; and blender [catboost, lightgbm, xgboost] that is a complex machine learning model containing catboost, lightgbm, xgboost models) and allows for predicting the compatibility of formulation ingredients.

Ingredients of the nasal form formulation were introduced in pairs into the system interface using the Simplified Molecular Input Line Entry System (SMILES), and their compatibility was checked. A platinum-platinum-rhodium thermocouple to heat the samples in aluminum crucibles (*from 15 to 250 °Ñ*) and á-Al_2O_3 as a reference substance (*the heating rate was 10° Ñ/ min*) were used for thermogravimetric studies (Shimadzu DTG-60, Japan) to confirm the compatibility of the formulation ingredients.

In addition to assessing the compatibility of ingredients, this method made it possible to additionally characterize the temperature regime for manufacturing the formulation. The individual API were studied including Ademol and other ingredients, such as glycerin, hydroxyethylcellulose (HEC), benzalkonium chloride, and the manufactured final nasal dosage form without active ingredients, and dosage form with Ademol. The mass of the studied samples ranged from 16.08 mg to 73.29 mg.

Rheological studies of the nasal form with Ademol were conducted using an MCR 302 rheometer (Anton Paar GmbH). As a measuring device, coaxial cylinders CC27/T200/SS were used at a temperature of 29-± 0.5°Ñ 33 ± 0.5° Ñ, 37 ± 0.5° Ñ, which makes it possible to determine the characteristics of the structural and mechanical properties of the nasal form as well as offer rational dosage form packaging. As a result, the prescription for the extemporaneous preparation of an intranasal dosage form for the cerebroprotective agent Ademol was created. In addition to the API, it also included the plasticizer glycerin, the mucoadhesive component HEC and the antimicrobial component benzalkonium chloride.[7] The research was carried out at the Department of Experimental Pathophysiology and Functional Morphology in the Training Medical and Laboratory Center of ZSMU.

The study was carried out on Wistar rats of both sexes, aged 10-12 weeks, weighing 170-230 g (n=29), obtained from the nursery of the Institute of Pharmacology and Toxicology NAMS of Ukraine. The cages with the animals were kept in separate rooms with a standard lighting regime: 12 hrs of light and 12 hrs of darkness. Animals were

excluded from the study during quarantine, if they did not meet the standard criteria. The duration of the quarantine (acclimatization period) was 14 days for all animals. During quarantine, each animal was examined daily (behavior and general condition), and twice a day rats were observed in cages (morbidity and mortality). Before the start of the study, animals that met criteria for inclusion in the experiment were divided into groups using the randomization method. Animals not meeting the criteria were excluded from the study during quarantine. The experimental animals were kept on the same diets under normal vivarium conditions. All manipulations were carried out in accordance with the regulation on the use of animals in biomedical experiments (Strasbourg, 1986, as amended in 1998-2001).

To facilitate subsequent experimental studies (*open field test*), animals were kept in hands for 2-3 min for 5 days before the experiment. Anesthesia with Thiopental sodium (40 mg/kg) intravenously into the tail vein was carried out by a 26G needle.

Experimental animals were subjected to suboccipital puncture under general anesthesia. The modified SAH model with a single injection of autologous blood based on the method of Dudhani R.V. et al. [11] was developed and used. Blood (0.2 ml) was taken from the tail vein with a heparin syringe. Then a large occipital cistern was punctured and 0.2 ml of autologous blood was injected into the subarachnoid space. The hole was closed with dental wax. Sham-operated animals underwent anesthesia and other surgical procedures without the introduction of autologous blood. The investigated agents were administered once a day at the same time: Ademol –intranasally at a conditionally effective dose of 2 mg/kg for 7 days; Nimodipine (Nimotop®, Bayer, Germany) – intraperitoneally 30 mcg/kg for 7 days. Animals in the control groups received equivolume quantities of solvents.

The open field test, based on subjecting an animal to an unknown environment whose escape is prevented by surrounding walls, was used to evaluate locomotor and search activity in animals on the 7th day after the SAH using the arena of our own production with dimensions of 80x80x35 cm, as indicated earlier.[12]

The experimental rat was seated with its muzzle against the wall and was given free access to movement (8 min). The total distance that the rat walked (cm), the sum of the activity of all movements (cm^2/sec), the structure of activity (inactivity, low activity, high activity, %), the number of ascents and visits to the center, approaches to the center, the distance traveled near the wall and to the central area of the arena were recorded (cm, %); movement speed, delay time before entering the center, number of grooming acts and number of defecation acts were estimated.

In the course of the study, we excluded (chamber of conditioned reflexes) the influence on the rat all possible stimuli as well as the presence of the experimenter during the period of registration. Registration of rat movements in the open field was performed using a SSC-DC378P color video camera (Sony, Japan).

The data were analyzed using the Smartv 3.0 program (Harvard Apparatus, USA). Statistical analysis of the experimental data was performed using the Microsoft Excel 2016 program with the AtteStat 12 statistical processing package. To assess the significance of differences in the study groups, the Kruskal-Wallis test with the Dunn's post hoc correction was used. Differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Simulation of SAH led to a decrease in the total activity of animals (by 3 times) and a decrease in the total path of movement (by 2 times). The experimental SAH also increased the duration of the path along the periphery (near walls and corners) by 2.3 times, and reduced the distance traveled before crossing the periphery-center (shadow-light) border by 2.43 times whereas the distance traveled in the illuminated center decreased by 3.8 times. Animals were characterized by inactivity, anxious-aggressive behavior, and poor orientation in space.

The simulation of the SC led to a significant increase in inertia when moving from the dark field of the arena to the illuminated part by 2 times indicating a decrease in locomotor and search activity as well as an increase in anxiety and fear. On the 7th day after SAH the duration of inactivity near the wall increased by 1.67 times indicating inhibition of exploratory activity and

Table 1. The effects of the investigated drugs on the behavior and exploratory activity of rats in the open field test on the 7th day after SAH

Characteristics	Subarachnoid hemorrhage (Control) (n=5)	Nimodipine (n=7)	Ademol-gel (n=7)	False-operated animals (n=10)
General activity, cm^2/s	12836.20 ± 1354.02[1]	13736.57 ± 1843,07[1]	23497.03± 9883.61	39592,10 ± 3692,40
Short grooming acts	1.00 ± 0.00[1]	2.29 ± 0.76[1*]	3.29 ± 1.70[1]*	7.10 ± 0.57
Defecation	0.80 ± 0.84[1]	1.71 ± 0.76*	2.14 ± 1.07*	2.80 ± 0.42
Distance the border-periphery zone, cm	480.42 ± 125.47[1]	553.32 ± 190.97[1]	280.66 ± 53.12*[2]	207.36 ± 50.63
Distance from the periphery to the center, cm	1684.07 ± 739.23[1]	1792.67 ± 836.27[1]	2618.32 ± 1662.85	4103.54 ± 555.48
Distance in the center, cm	66.84 ± 19.45[1]	120.32 ± 13.57[1]*	161.21 ± 54.58*	249.67 ± 41.27
Total distance, cm	2026.04 ± 696.53[1]	2495.38 ± 557.79[1]	2751.73 ± 356.56[1]	4148.40 ± 507.26
Duration of high activity, %	9.24 ± 1.40[1]	12.94 ± 4.33	17.44 ± 2.71*	18.79 ± 2.16
Duration of low activity %	40.70 ± 9.88[1]	37.13 ± 13.62[1]	50.56 ± 9.10	61.99 ± 7.95
Total duration of immobility (sec)	230.25 ± 73.99[1]	108.33 ± 30.21[1]*	76.73 ± 20.93*	50.30 ± 9.24
Maximum moving speed in the center (cm^2/s)	29.59 ± 13.28[1]	178.31 ± 40.09*	199.52 ± 45.16*	169.08 ± 33.82
The first delay when entering the center, sec	92.04 ± 8.17[1]	79.39 ± 13.87[1]	68.12 ± 12.62*	47.42 ± 6.01
The duration of inactivity in the internal periphery, %	52.00 ± 4.53[1]	40.14 ± 5.49*	33.43 ± 6.21*	31.40 ± 3.50
Duration of inactivity periphery border – center, %	32.00 ± 5.05[1]	38.14 ± 24.75	19.86 ± 5.30	13.80 ± 9.91
Rearing	832.35 ± 69.91[1]	734.17 ± 78.34[1]	476.00 ± 158.95*	278.81 ± 50.41

Note: * – statistically significant difference ($p<0.05$) compared with the control group;
[1] – statistically significant difference ($p<0.05$) compared with the group of false-operated animals

depression development. In animals after SAH, the number of rearings, a common measure of activity and exploratory behavior, increased by 3 times, immobility increased by 2.46 times when passing from the periphery to the center of the arena (which can be regarded as an increase in anxiety, fear and disorientation in animals with SAH). The speed of passing the path in the illuminated center of the arena decreased by 5.86 times.

The number of free rearings did not change when modeling the ICG, but the number of rearings at the wall increased. A decrease in the number of short grooming acts against the background of a constant number of long-term grooming testified to increased anxiety, excitability and irritability of the animals, as well as their discomfort and depression. A decrease in short grooming by 7 times and defecation acts by 3.5 times also testified to a decrease in high activity. The decrease in high activity noted above in the animals of the control group testified to the low emotionality and excitability of the animals. The introduction of Nimotop immediately after the animals came out of anesthesia as well as intranasal application of a new dosage form of the neuroprotector Ademol-gel had different effects on behavioral reactions, cognitive and exploratory functions, as well as emotional status of animals (table).

Ademol-gel reduced the distance traveled in the border-periphery zone, and significantly exceeded the effect of nimodipine in this indicator (by 2.3 times). Ademol-gel increased the number of short grooming and defecation acts by 3.3 and 2.67 times, respectively, and reduced the number of rearing by 1.75 times. All this indicated a decrease in anxiety, depressive behavior, and aggressiveness, and an increase in empathy in experimental animals.

Intranasal administration of Ademol-gel to animals with SAH led to the restoration of the active component of the research activity. This was evidenced by an increase (significantly by 1.88times) in the duration of high activity of animals such as examination of objects above the floor of the arena and the number of jerks near the wall. This fact indicated the positive effect of Ademol-gel on the cognitive functions of the central nervous system.

Animals treated with Ademol-gel for 7 days after SAH also showed better spatial orientation and freer movement in the illuminated part of the arena. Thus, the speed of their movement in the illuminated center increased by 6.8 times. The time of general immobility in the Ademol group also significantly (by 67 %) decreased, while the indicators of general activity and the total distance traveled did not change significantly in the nimodipine group.

The results of the research showed that experimental SAH led to a significant violation of orienting-exploratory behavior, as evidenced by the indicators of the open field test. Thus, in animals after SAH modeling, a decrease in high and low activity was noted, which indicated the suppression of the exploratory function of the central nervous system, as well as the formation of anxiety and excitability. The lack of movements aimed at mastering a new environment, a decrease in high and low activity in animals may indicated a decrease in cognitive abilities.

Our experimental results do not contradict the data of other researchers on the molecular and biochemical mechanisms of cognitive-mnestic disorders of the CNS after TBI and subarachnoid hemorrhage, cerebral stroke. Experimental SAH leads to persistent disturbances in memory, orientation, research and cognitive activity of animals, the appearance of irritability, lethargy, fear, anxiety, disorientation, aggressiveness.[13-14]

Cognitive-mnestic disorders after SAH are formed in response to transmitter autocoidosis, hyperactivity of neuronal nitric oxides synthase (nNOS) and inducible nitric oxides synthase (iNOS), increased production of reactive oxygen species, energy deficiency, lactic acidosis, mitochondrial dysfunction, increased Fe-dependent reactions of free-radical oxidation, leading to the initiation of apoptosis of neurons in the zone hippocampus and sensorimotor cortex.[15] Experimental animals treated with ademol-gel after SAH were significantly more mobile, and showed increased interest in the environment. Animals after SAH treated with Ademol-gel showed fewer signs of fear and anxiety compared to the control group, as evidenced by a decrease in the time of the first delay when entering the center by 26 %.

The pharmacological effects of Ademol are associated with its ability to reduce excitotoxicity and maintain the functional activity of hippocampal

and sensorimotor cortex neurons by reducing the increased excitability of NMDA receptors and modulating the NMDA polyamine site.[16] Professor A.A. Khodakovsky and co-authors also demonstrated the presence of anxiolytic properties in Ademol due to its ability to increase affinity for GABA receptors, leading to a decrease in fear and anxiety.[17-18] Ademol can provide endotheliotropic action by reducing platelet aggregation, increasing the expression of nitric oxide synthases. It was also revealed that adamantane derivatives had mitoprotective and energotropic action.[19] A less pronounced positive effect of pharmacotherapy on the psycho-emotional behavior and motor-search activity of rats with SAH treated with nimodipine was noted. Animals treated with nimodipine (Nimotop®, Bayer, Germany) were characterized by inactivity, greater anxiety and aggressiveness. In the nimodipine group, no significant changes in motor and search activity were recorded compared to the control group. Some indicators of anxiety were worse, and indicators of exploratory activity were significantly lower in the Nimotop group than in the group of animals after SAH treated with Ademol-gel.

CONCLUSION

1. The behavior of animals after SAH was characterized by a significant decrease in total activity by 3 times, a decrease in the distance traveled by 2 times and an increase in inertia when moving from the dark field to the illuminated part of the arena by 2 times, which indicated a significant decrease in motor and search activity. The number of unsupported rearings (in which the animal freezes without touching the walls of the arena) did not change, but the number of unsupported rearing (in which the animal leans on the walls of the arena) increased; the number of short groomings decreased against the background of a constant number of long groomings. Such behavior indicated an increase in excitability, anxiety, irritability of animals, the development of severe discomfort and depression in them.

2. In animals with severe neurological deficit after SAH Ademol-gel 2 mg/kg intranasally had a positive effect on the emotional status and behavior of animals in the open field test, as evidenced by a decrease in anxiety, indicators of aggressive and depressive behavior against the background of an increase in comfort and the formation of empathy. The motor and exploratory activity of animals with SAH also quickly returned to normal after the administration of Ademol-gel.

3. In rats in the acute period of SAH, Nimodipine (Nimotop®, Bayer, Germany) intraperitoneally at a dose of 30 μg/kg, as a primary neuroprotector, realized its action aimed solely at maintaining the viability of neurons, without having a significant effect on animal behavior and cognitive deficits.

4. The experimental results obtained confirm the prospectivity and effectiveness of administration of a new dosage form - Ademol intranasal gel in the management of TBI to accelerate the elimination of cognitive deficits and restoration of motor activity.

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Conflict of Interest

The authors declare no conflict of interest, financial or otherwise with this work.

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