COGNITIVE IMPAIRMENT IN PRIMARY AND REPEATED HEMORRHAGIC STROKE AND THEIR CORRECTION WITH VERAPAMIL

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Annotation. *The article is devoted the actual problem of modern medicine – pathogenesis and treatment of hemorrhagic stroke. In order to analyze the influence of Verapamil in preventing cognitive disorders under stroke a local hemorrhagic stroke in 60 rats was simulated there. Assessment of the cognitive function was performed by the Buresh method. We found out that rats after stroke were observed to show memory disturbances. Under stroke and after Verapamil injections the rats had a gradual decrease of disorders in primary and repeated intracerebral hemorrhage. These data suggest inhibition of calcium-dependent neurodegenerative processes in the rat cortex and cognitive disorders under Verapamil injections after stroke.*

Keywords: *stroke, cognitive function, Verapamil*

Introduction. The cognitive activity of the organism is a set of complex coordinated higher levels of brain functions – memory, intelligence , providing the process of environment perception and vital activity adaptation. Quite often there are evident disturbances of cognitive functions, which reasons can be the factors, various in etiology and pathogenesis, [5]. Among these factors cerebral hemorrhage into the internal brain capsule (hemorrhagic stroke – primary and repeated) occupy one of the leading places in the structure of cerebrovascular diseases and their complications, the result of which, most often, is impairment of the CNS cognitive functions [6]. The problem of hemorrhagic stroke, its complications (catastrophic memory disorders and learning ability disturbances) and medicinal treatment is particularly urgent in modern medicine. The action and duration of the use of innovative medicines are tightly connected with the extent of pathophysiological and morphological changes of the nervous system under primary and repeated strokes [2]. Therefore, the profound studying of the leading mechanisms of development of the functional and morphological changes under primary and repeated hemorrhagic stroke and the corresponding research of new underlying disorders of the cognitive functions is actual now. In modern neuropharmacology a significant amount of medicines, used for correction of the disturbances, which develop as a result of hemorrhagic strokes (nootropic preparations, vaso- and neuroprotectors, blockers of calcium channels), various in the mechanisms of action have been developed, however, their efficiency in pilot studies is subjected to criticism by many authors. Clinical efficiency is proved only for some of them (for example, for cyticholine) [7].

Systemic development of the pathophysiological disorders causing neurologic impairment in the conditions of acute stroke and under chronic brain ischemia includes step-by-step impairment formation, where each stage is characterized by certain cellular and molecular changes: conduction of nerve impulses by neurons and microcirculation

disorders, oxidizing stress and hyperproduction of mediators, secretion changes and neurotransmitter reception, apoptosis and necrosis development.

At the same time, one of the main reasons for diffusion functional impairment under local stroke is a secondary affection of the white brain substance. It is considered that the rate of metabolic processes in the white substance is only a little lower, than in the cerebral cortex, and structurally functional disorders of the white substance can take place even under short ischemia. Energy insufficiency under ischemia leads to disorders of ionic gradient maintenance, depolarization of membranes and, finally, results in calcic cytotoxicity, which activates Ca^{2+} dependent enzymes provoking irreversible damages of glia and axons of the white substance.During a hemorrhagic stroke in nerve cells acute energetic deficit and dysfunction of ionic channels develops: Na^{+}, K^{+} and Ca^{2+} transport through a plasma membrane is getting broken, provoking neuron depolarization, activation of proteolytic enzymes, causing apoptosis and necrosis of damaged cells [6]. According to modern conceptions, the pharmacological correction of the raised calcic current in cytoplasm of neurons is provided by inhibition of Ca^{2+}- channels, NMDA-and AMPA receptors. It has been established that some inhibitors of Ca^{2+}- channels significantly improve a cognitive state after stroke patients [5].

As a variant of experimental therapy, experimental animals were injected with a blocker of calcium channels Verapamil that is an antiarhythmic, hypotensive and antianginal drug, which is applied for stroke prevention, first of all ischemic stroke, but there is an insignificant number of works, devoted to application of calcium antagonists for the patients after hemorrhagic stroke. Verapamil has its blocking effect on calcium channels (effects from the internal side of a cell membrane) and reduces the transmembrane calcium current that, perhaps, changes the level of brain affection, blocks the calcium mechanism of cell damaging. It slightly affects alpha adrenoceptors and sodium channels as well. In the works it is shown that high doses of intra arterial Verapamil introduction provoke some increase of intra cranial pressure and a decrease in cerebral perfusion pressure, with the subsequent increase in the level of glucose. The results of the conducted trials specify that blockers of calcium channels can be perspective for future clinical applications under primary or secondary brain injuries [by Merkel MJ, 2 008]. But the efficiency of Verapamil application for correction of mnestic and cognitive functions of the organism in the conditions of primary and repeated hemorrhagic strokes has not been investigated that defines the relevance of this work.

Research objective: to estimate changes of the cognitive function of rats in the conditions of experimental primary and repeated hemorrhagic stroke and the influence of Verapamil as a means of correction of calcium- dependent degenerative processes.

Materials and methods of research. The pilot study has been conducted with 60 male rats (the average weight of 210-230 g). The rats were kept in standard conditions of the vivarium, without restrictions in a diet. All animals were divided into 3 main groups: the I-st group - control (A- intact and B- pseudo-operated), the II-d group - animals with primary hemorrhagic stroke, the III-rd group – the animals with repeated hemorrhagic stroke. Experimental groups II and III, in their turn, were divided into subgroups for determination of the efficiency of pharmacological Verapamil correction. So, group II consisted of subgroups IIA (primary hemorrhagic stroke without correction) and IIB (primary hemorrhagic stroke with Verapamil correction); respectively, IIIA – the animals

with a repeated hemorrhagic stroke without correction, IIIB – the animals with a repeated stroke with Verapamil correction.

Hemorrhagic stroke simulation was carried out according to a stereotactic method by means of mechanical destruction of the brain tissue in the internal capsule area within the right hemisphere by means of a mandrin-knife with some additional introduction of 0,1 ml of auto-blood into a zone of destruction. For repeated stroke simulation experimental rats were subjected to intracerebral hematoma reproduction for the second time on the same coordinates, stated above, in 1 month after carrying out a primary procedure [4].

After the operation groups IIB and IIIB were taking Verapamil during the term of 10 days in the dose of 0,1 mg/kg (intraperitoneal introduction).

Assessment of the level of cognitive functions in the rats of experimental groups was carried out by methods of "conditional reflex reactions of the passive avoidance" (CRPA) according to Ya. Buresh [1]. CRPA was represented by means of the experimental installation consisting of two chambers: a lighted one, the size of 25x25 cm and a dark one, the size of 25x25 cm with the electroconductive floor. The rat was put into the center of the light chamber, with its tail directed to the hole of the partition. The animal, having found a hole in the partition, passed from the lighted chamber to the dark one. Within 3 minutes the time of the rat's staying in the dark chamber and time of the first visit are being registered. After the time termination, when the animal was in the dark chamber, it was subjected to electrical nerve stimulation through the floor (50 Hz, 1,5mA) until the rat did not pass to the light chamber. If within 10 seconds the animal did not come back to the dark chamber, it was put back to the cage, if comes back into the dark chamber irritation was caused repeatedly. For assessment of CRPA maintenance and reproduction the animals were tested in 24 hours.

An ability to memorization was counted according to a difference of the latent time (LT) of coming into a dark compartment (chamber). Skill retention were specified taking into account latent time changes of the rat's coming into a dark chamber; also the percentage of the animals with amnesia - the rats, not remembering the danger of coming to the dark chamber.

In our opinion, in the acute period of neurologic deficiency phenomena for excluding of any effect on the test quality, when rats perform CRPA on the one hand, and, on the other hand, considering the ability of rats to memorize a memorial trace within three weeks. – studying of the developed experience reproduction by animals on the 14^{th} day is more informative.

Experiments were made according to the methods and requirements of the SEC of Health Ministry of Ukraine and with the requirements and norms of "The European convention on protection of the vertebrate animals used for experimental and other scientific purposes" (Strasbourg, 1986) [3].

Results of research and their discussion. The results of impact of primary and repeated hemorrhagic stroke on the cognitive function of rats in the experiment, correction of disorders of these functions by Verapamil and dynamics of training and memory disorders of rats in the conditions of CRPA reproduction are given in table 1.

Carrying out the CRPA test in the intact group of animals did not show any essential difference with the latent time indicator before a surgical intervention and made 149 ± 20,7 s (20%). After CRPA reproduction the latent period did not change that testified to

formation of memory (reflex) of the "dangerous" zone in the carried-out test and the lack of cognitive disorders well as.

It is known that during the first week after animals' training in the experimental installation the rats from the group of pseudo-operated animals carry out CRPA and are adapted to test conditions for memorization of the "dangerous" dark chamber; it was repeatedly confirmed in pilot studies. On the 14^{th} day the latent period of coming into the dark room in this group of animals made 131,5 ± 24,7seconds, 30% did not fill the test, that testifies to an expected characteristic of a memorial trace in animals with amnesia. It is known, that such adaptation reactions of rats in unfamiliar conditions appeared to be a congenital stereotype of behavior, and memory formation – some acquired consolidated experience – the more complex stereotype. Experience as an ability for training and acquirement of individual skills, does their behavior more flexible and adaptive, and their realization are necessary for a survival and is directly connected with cognitive activity.

In the group with primary hemorrhagic stroke to the end of the 2-d week the latent time of coming to the dark room made 92,5 ± 23,9 seconds that testified to memory disorders, that is 21,7% less in comparison with the latent time to the operation of the pseudo-operated animals. Thus, 60% of animals came into the dark chamber in the group with primary hemorrhagic stroke, in comparison with control that was 30% more than in the control group.

In the group with primary cerebral hemorrhage in the conditions of correction of this state with Verapamil the latent time of animals' coming into the dark chamber made 100 ± 22,1 seconds on the 14^{th} day in the experiment. Thus, 55% of animals came into the dark chamber came into a dark chamber under Verapamil correction of primary hemorrhage. It testifies about the insignificant (doubtful) efficiency of Verapamil application under HS.

The latent time of coming into the dark chamber on the 14^{th} day in the group of repeated cerebral hemorrhage averaged 62 ± 20,3 s in comparison with the value before the operation. When determining the percentage of animals, remembering about the electric current at the end of the 2-nd week made 20 percent in the group of animals with amnesia (80% of animals came into the dark chamber) that testifies to essential disorders of the CNS cognitive functions and reliably higher in comparison with a group of animals with primary stroke.

The rats with a repeated hemorrhagic stroke taking Verapamil within 10 days after repeated hemorrhage reproduction in the internal capsule of the brain were observed to show some reduction of time of the animals' coming into the dark chamber (the average time of staying in the chamber made 84 ± 26,1 seconds). The quantity of the animals with amnesia from the total of animals of this group made 60%. That testifies to the positive influence of Verapamil for correction of cognitive memory disorders under repeated cerebral hemorrhages in the experiment.

Table 1.

The values of CRPA reproduction in rats in the conditions of experiment on the 14^{th} day (n=60)

Group of animals	Latent time , s.	Number of animals with amnesia, %
Intact (group IA) **(n=10)**	149±20,7	20%

Pseudo-operated (group IB) **(n=10)**	131,5±24,7	30%
HS (group IIA) **(n=10)**	92,5±23,9	60%
HS+Verapamil (group IIB) **(n=10)**	100±22,1	60%
RS (group IIIA) **(n=10)**	62±20,3*	80%
RS+Verapamil (group IIIB) **(n=10)**	84±26,1	60%

Note: HS - hemorrhagic stroke, RS - repeated stroke * - reliable for intact rats ($\leq 0,05$).

That is, a reliable statistical difference between groups of comparison II and III in the conditions of CRPA reproduction in the carried-out series of experiments has not been determined, however, a tendency to reduction of the term of coming into the "dangerous" dark chamber that is memory impairment was noted. There is statistically significant reduction of the time of memorial trace realization in group IIIA in relation to group I.

Thus, the results of the conducted researches showed that in the rats with unilateral hemorrhagic stroke memorial disorders are authentically formed.

Pathophysiological researches showed that the use of the antagonist of Verapamil calcic channels authentically reduces cognitive impairment progressing at the heart of which there is prevention of calcium-dependent neurodegenerative processes developing against the background of ischemic lesion under a local hemorrhagic stroke. Functionally it is shown in reduction of the cognitive deficiency symptoms. It is known that excess accumulation of free calcium in cytosol conducts to destruction of intracellular structures, degeneration and the death of nerve cells [5,6]. Free calcium ions activate a number of nucleases and proteinases that leads to disorders of the structure and functions of proteins and nucleinic acids that makes reparative processes impossible. In the conditions of such disorders using of antagonists of calcic channels reduces calcium influx and inhibits neurodegenerative processes [8]. Using of Verapamil under such circumstances allowed to prevent progressing of these changes.

Conclusions. 1. In the conditions of experimental intracerebral post-traumatic hematoma (hemorrhagic stroke) cognitive impairment in the form of memory disorders and realization of a memorial trace authentically develop.

2. The revealed changes of cognitive impairment in rats with primary and repeated hemorrhagic stroke testify to considerable memory disorders on the 14^{th} day after hemorrhage that is connected with the development of the progressing neurodegenerative changes of the cerebral cortex.

3. Verapamil authentically ($p \leq 0,05$) reduces cognitive impairments in rats with primary and repeated strokes that is reflected in a gradual increase in the latent time of coming into the dark "dangerous" chamber.

4. When using Verapamil as a neuroprotector (mnestic protector) under repeated hemorrhage, some reduction in the quantity of animals with amnesia by 20% is noted, that testify to a probable efficiency for correction of memory disorders as a consequence of repeated hemorrhage in the internal capsule.

Prospects of further researches. Further researches of the pathogenesis of cognitive impairment in case of treatment by other medicines of the group of Ca^{2+}-blockers are planned.

Literature:

1. Buresh Ya. Metodiki i osnovnyie eksperimentyi po izucheniyu mozga i povedeniya / Ya. Buresh, O. Bureshova, D. P. Hyuston — M. : Vushaya. shkola., 1991. — 399 p. (In Russian)
2. Gorbas I. M. Faktory ryzyku mozkovogo insultu: poshirenist, dynamika, kontrol / I. M. Gorbas / Sudinni zahvoryuvannya golovnogo mozku. — 2010. — № 5—6. — P. 22—25. (In Ukrainian)
3. Etika likarya ta prava lyudyni: polozhennya pro vikoristannya tvarin u bIomedichnih doslIdah // Eksperim. ta klInIch. fIzIologIya I bIohImIya. — 2003. — № 2. — P. 108—109. (In Ukrainian)
4. Metod modelirovaniya lokalnogo krovoizliyaniya v razlichnyih strukturah golovnogo mozga u eksperimentalnyih zhivotnyih / A.N. Makarenko, N.S. Kositsyin, N.V. Pasikova [et. al] // *Zhurn. vyissh. nervn. deyateln*. — 2002. — V. 52, № 6. — P. 765—768. (In Russian)
5. Yahno N. N. Kognitivnyie i emotsionalno-affektivnyie narusheniya pri distsirkulyatornoy entsefalopatii / N. N. Yahno, V. V.Zaharov // RMZh.— 2002. — V. 10. — P. 539—542. (In Russian)
6. Bano D. Ca2+ Signals and neuronal death in brain ischemia. glutamate-independent calcium toxicity / D. Bano, P. Nicotera // Stroke. – 2007. – Vol. 38, № 2. – P. 674−676.
7. Chen G. J. The effects of calcium channel blockers in the prevention of stroke in adults with hypertension: a meta-analysis of data from 273,543 participants in 31 randomized controlled trials / G. J. Chen, M. S. Yang // PLoS One. – 2013. – Vol. 8, № 3. – P. 57854.
8. Cho H. J. Efficacy and safety of oral citicoline in acute ischemic stroke: drug surveillance study in 4,191 cases / H. J. Cho, Y. J. Kim // Methods find exp. clin. pharmacol. – 2009. – Vol. 31, № 3. – P. 171−176.
9. Lee J.E.Edema after stroke, one of major brain injuries / J.E.Lee, J.H.Kim, J.Y.Kim // International Journal of Stroke. – 2008. – Vol.3, № 1. – P. 173.