UDC 616.831-005.1

RATS CORTEX OF BRAIN INJURY AFTER EXPERIMENTAL MODELING OF INTRACEREBRAL HEMORRHAGE

T.M. Oliinyk[1], S.I. Savosko[1], V.M. Bibikova[2]
[1]Bogomolets National Medical University, Kyiv, Ukraine
[2]Dnipropetrovsk Medical Academy, Dnipropetrovsk, Ukraine

Summary. *The paper describes the study of structural and metabolic changes in the brain cortex after hemorrhagic stroke and effects of increased blood pressure on the course of these disorders. For the purpose of studies we conducted histological study, catalase and SOD levels, ERK kinase levels and degree of DNA fragmentation. The structural and metabolic changes in rat cerebral cortex were investigated at different degree of hemorrhagic stroke. Intracerebral hemorrhage transformation, perifocal edema and enzymatic dysfunction increased at epinephrine application before stroke modeling. There was significant difference in the other parameters when compared to controls and mild form of stroke. Superoxide dismutase levels in motor cortex were found to be significantly reduced by 23,6% with a tendency to decrease in the group of modeling stroke after high doses adrenaline application. The catalase levels respectively decreased by 15,4%. The degree of DNA fragmentation in perifocal brain cortex did not differ significantly between the two models of hemorrhagic stroke and developing by necrosis type rather than apoptosis. These data indicate that ischemic injury in brain tissue decreased antioxidant potential that is a factor of progressive degenerative processes in perifocal brain cortex. The results indicate considerable oxidative stress in hemorrhage stroke and substantially increase in high blood pressure. These data can be used for research hemorrhagic stroke pathogenesis and determine the action of neuroprotective, metabolic and antiedemadrugs.*
Key words: *stroke, brain edema, blood pressure, enzyme dysfunction.*

Introduction. Hemorrhagic stroke, frequent vascular disease of the brain, is a significant medical and social problem. Previously, were developed a method of local hemorrhage stroke (intracerebral hemorrhage, ICH) [9], which is now widely used by various specialists, pharmacologists, pathophysiology [1]. In this paper, we propose a model of severe cerebral stroke. The main requirement for its development was the best match the flow of this disease in patients that developed on the background of chronic previous disease, in particular hypertension [7, 8].

At present, various methods for modeling of acute hemorrhagic stroke are proposed [12]. Hemorrhage caused progressive introduction in the region of the internal capsule autologous blood (2 ml), accompanied by the formation in pigs is not standardized ICH. The modeling process is accompanied by rapid damage to large areas of the brain, the destruction of the basal cell structures and, more rarely, the pathways of white matter and cerebral cortex. Moreover, this and similar experimental models are characterized by a high percentage of mortality of animals, which makes specified pattern unsuitable for use in chronic experiments. Besides modeling techniques of acute hemorrhagic stroke mainly designed for large animals,

while modern screening studies suggest use small laboratory animals (rat, mouse, etc.) [3, 4, 5, 11].

The aim of this study was to develop a model of local stroke is similar in pathogenesis, scope and extent of brain damage on the background of high blood pressure.

Material and methods.

Animals. The male Wistar Kyoto (WKY) rats (200-250 g) were used for the study. The animals were housed under well-controlled conditions of temperature (22,0±2,0^{o}C), humidity (55,0±5,0%) and 12h/12h light-dark cycle. They were allowed free access to standard rodent pellet diet and drinking water. The food was withdrawn 12 h prior to surgical procedure; however, water was allowed ad libitum. The experimental manipulations were carried out in accordance by University Ethics Committee, Regulations on the animal use of in research biomedical research, European Convention for the protection of vertebrate animals used for experimental and other scientific purposes.

Experimental protocol.

The male WKY rats (200-250 g) were divided into 3 groups of six rats each.

Group 1: Control (n=10) – injected i.p. (0,2 ml)sterile Sodium Chloride 0.9% (Normal Saline).

Group 2: ICH (n=15) – modeling right hemisphere intracerebral hemorrhage.

Group 3: Adrenalin+ICH (n=18) – modeling right hemisphere intracerebral hemorrhage after enhance high blood pressure. Adrenalin (1,5-1,8 mg/kg, i.p.) was injected once before 10 minutes to the experiment procedures.

Modeling arterial hypertension and hemorrhagic stroke.

Surgical procedure was performed according to method of Makarenko et al. [9]. The rats were anaesthetized by thiopental sodium (40 mg/kg, i.p.) and supplemented as needed.Modeling intracerebral hematoma in anesthetized animals was performed as a result of mechanical destruction of tissue inside the internal capsule (capsulainternadextra, L=3,5-4,0; H=6,0; AP=0,6-1,0) [10]. In internal capsule using stereotaxic instrument introduces prepared mandren knife. Direct modeling process is carried out by rotating movements declined by 4-6 and mandren sharpened knife with a view focused primarily damage the blood vessels in this part of the brain and the subsequent introduction of the zone of destruction 0,2 ml previously received autological blood animals. After surgery and complex sequential ICH modeling wound manipulation in the region of the skull sewn tightly polyamide filaments 2 USP (Olympus, Ukraine) and then treated with 5% alcoholic solution of iodine.

Enzyme activity. Catalase and superoxide dismutase (SOD) activity was determined by generally accepted methods [2,6].

Brain weighed sample (100 mg) homogenized with an electric homogenizer Glas-Col (USA) in 1 ml of 0,05 M phosphate buffer with 0,1 mM EDTA (pH 7,6). Enzyme activity was determined in the supernatants obtained by centrifuging the homogenate at 10,000 g for 20 minutes, using known spectrophotometric methods using a spectrophotometer ìQuant, Bio-Tek, (USA).

Histopathology.

Ten days after ICH modeling animals were anesthetized with 50 mg/kg thiopental sodium intraperitoneally and perfused through the left ventricle with cold

4% paraformaldehyde in 0,1 M phosphate buffer (pH 7,4). The brains were immediately removed; coronal forebrain sections were cut at 15-ìm intervals using a cryotome and stained with Nissl method, H&E-stained method.

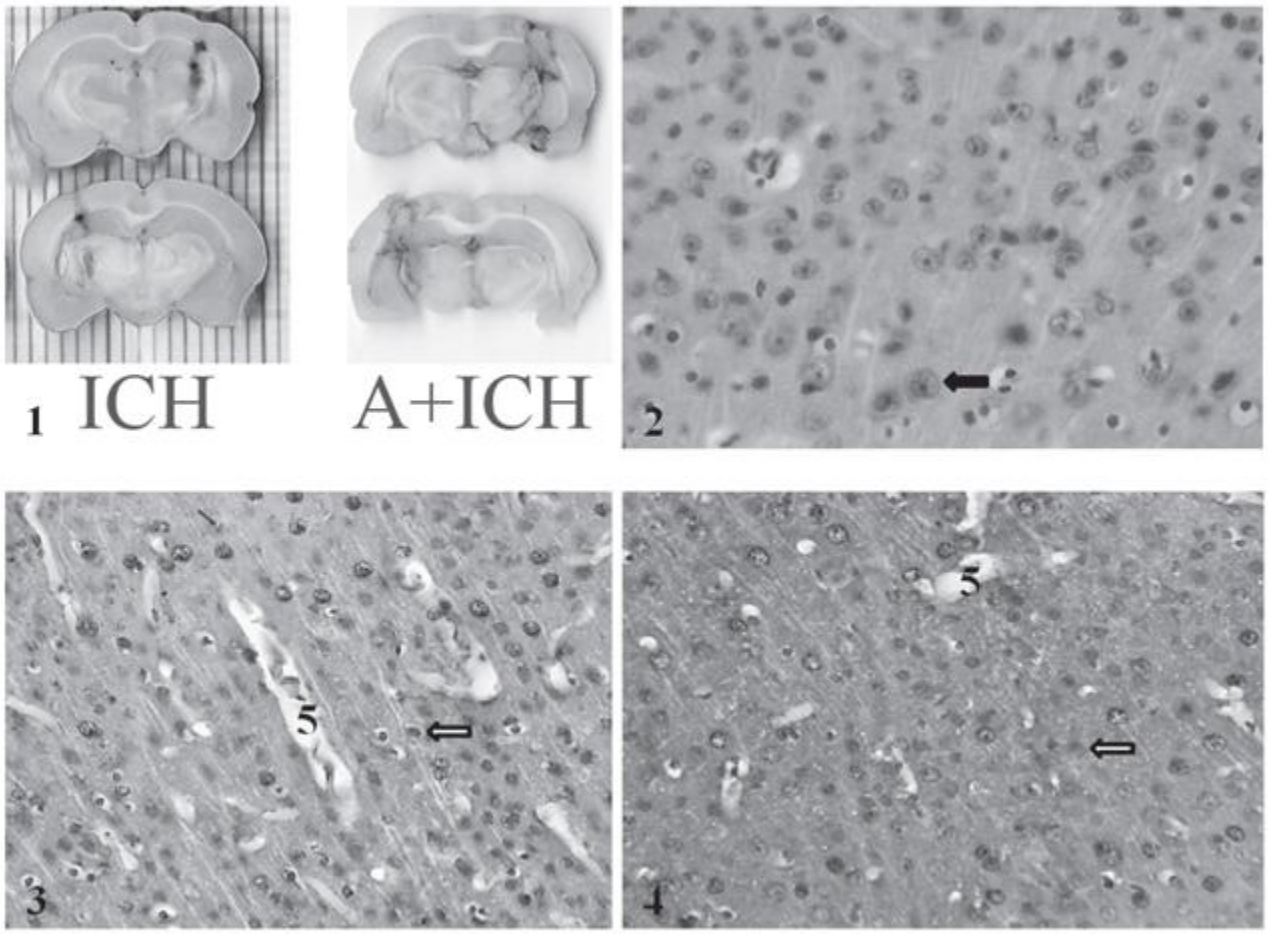

Figure 1. Histopathologic damage of perifocal brain cortex after 5 days of stroke. *Note: coronal sections (1) of rat brain through the frontal lobe show increasing degrees of histopathologic damage at adrenalin-induced acute hemorrhagic stroke. H&E-stained section shows no neuronal loss in control group (2); brain edema, neuronal cell loss (3); acute edema and neuronal necrosis (4); perivascular edema (5); normal (⬅) and necrotic neuron (⇦).*

Microscopic imaging.

Numerous photomicrographs were optically grabbed by Olympus Microscope (Olympus BX 51). Morphometric analyses were performed by using Carl Zeiss software (AxioVision SE64 Rel.4.9.1). For a quantitative analysis of changes in the brain cortex were determined following morphometric parameters: number of neurons per unit area ($1mm^2$), and the percentage of altered neuronal degenerative. Degenerative cells with signs of cytolysis, cariolysis cariopicnosis neurons with homogeneous stained acidophilic nuclei that do not contain nucleoli.

Statistical analysis. Data were presented as mean±SEM and statistical analyses were evaluated by software package «Statistica 12.0» («StatSoft», USA). Student t-test was used to parametric ordinal data; Mann-Whitney U-test was used to analyze nonparametric ordinal data. P value <0,05 is considered as statistically signicant.

Results and discussion.

The histological study observed polymorphic structural changes in brain cortex and subcortical cell formation. (Figure 1 B, C). There are some shrunken hyperchromic neurons, neurons with hypertrophy or cariopicnosis. Showing signs of swelling and significant pericellular edema (mainly in dendrites). In the motor cortex shows signs of activation of glial cells, this is accompanied by increased tinctorial properties of the cytoplasm in the form of a sharp hyperhromatosis. In pyramidal neurons clearly expressed central and peripheral destruction of basophilic substance. We also registered blood vessels with perivascular edema and stasis.

In corpus callosum we established apoptotic glial cells arranged randomly nerve fibers. White matter in this zone has pronounced swelling. In the ipsilateral internal capsule set gliosis and infiltration of polymorphonuclear neutrophils around hemorrhage.

In the group of animals that simulated hemorrhagic stroke after epinephrine application character of histopathological changes had similar trends, but has more than progressive brain edema and neuronal death in motor cortex. A more detailed comparison is imposed in the table 1.

Structural changes in cerebral cortex after stroke are manifestation of the pathological process, but do not provide a clear understanding of the pathophysiological and metabolic disturbances during ischemia. The analysis of enzyme activity of endogenous antioxidant system showed serious violations protective opportunities in nerve cells at hemorrhagic stroke. Biochemical research has shown that SOD activity in motor cortex decreased by 23,6% (p=0,007) with a tendency to decrease in the group of modeling stroke after high doses adrenaline application (Table 2, figure 2). The activity of catalase respectively decreased by 15,4%. These data indicate that ischemic injury in brain tissue decreased antioxidant potential that is a factor of degenerative processes progression in perifocal cortex.

Intracellular enzyme “switches” were also violations. For example, the activity of ERK kinase 1, an enzyme that combines the signals from cell receptors to cytoplasmic enzymes, decreased by 26,1% and 44,3%, and ERK kinase 2 – has not changed (Table 3). I.e., at severe hemorrhage intracellular signaling systems are more excited.

The degree of fragmentation of nucleic acids, such as DNA, in perifocal cortex homogenate did not differ significantly between the two models of hemorrhagic stroke (Figure 3).

Hypertensive intracerebral hemorrhage is caused by long-term high blood pressure (hypertension). At arterial hypertension pressure remains high over a long time period, disrupt blood vessel that can lead to stenosis or ICH. Hematoma causes swelling of brain tissue and leads to irreversible changes.

Comparative analysis of two models of hemorrhagic stroke showed that pre-high blood pressure when administered high doses of epinephrine impairs the structural and metabolic changes in the motor cortex of rats that cause harder degree of bleeding, which is confirmed by histological examination.

Conclusion. The animal models of local hemorrhagic stroke are maximal standardized and used to modeling ICH in other areas of rats brain. The model may be useful for evaluating the effectiveness of neuroprotective and metabolic drugs and experimental studies of pathophysiology of cerebral pathology.

Reviewer: professor V.G. Cherkasov

Table 1.

Histopathological changes in our proposed model of acute hemorrhagic stroke

Histopathological changes		Group	
		ICH	A+ICH
Acute neuronal injury	cases	15	18
	%	100	100
	se	++	+++
Gliosis	cases	8	5
	%	53,3	27,7
	se	++	++
Brain edema	cases	15	18
	%	100	100
	se	++	+++
Perivascular edema	cases	15	18
	%	100	100
	se	++	+++
Polymorphonuclear leukocytes	cases	7	14
	%	46,6	77,7
	se	++	+++

Note: se – semi-quantitative evaluation

Table 2.

Catalase and SOD activity in rat perifocal cortex after modeling intracerebral hemorrhage.

Group		SODu/mg	CAT mol/mg
1	Control	16,83±0,23	1,43±0,01
2	ICH	12,86±0,86	1,21±0,08
3	A+ICH	11,93±0,54	1,26±0,07
p-value		P_{1-2}=0,096 P_{1-3}=0,007 P_{2-3}=0,378	P_{1-2}=0,173 P_{1-3}=0,185 P_{2-3}=0,273

Note: values are expressed in Mean±SEM

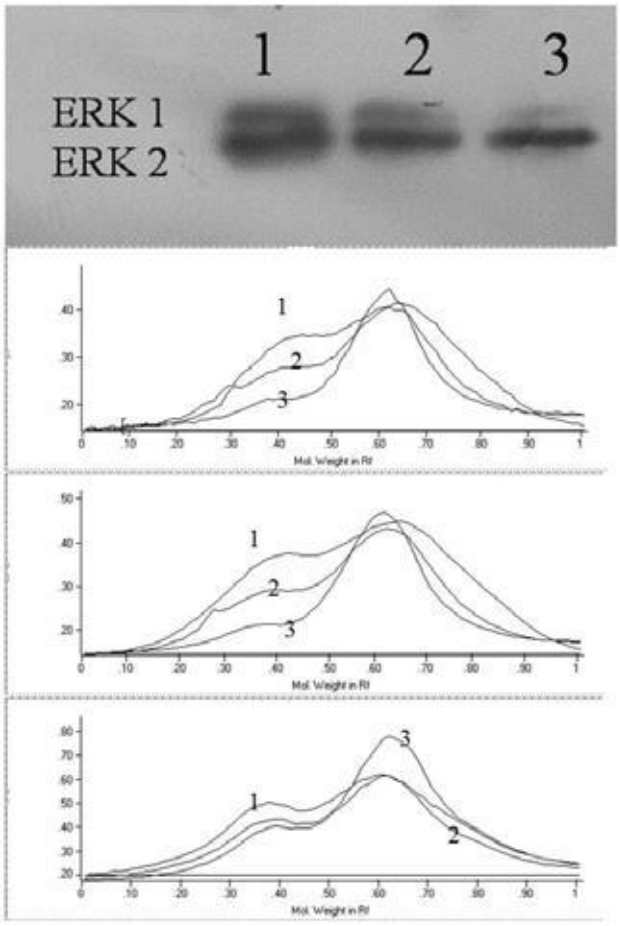

Figure 2. ERK 1/2 electrophoresis in the perifocal brain cortex after modeling intracerebral hemorrhage. *Note: 1 – control; 2 – ICH; 3 – A+ICH. Reduce ERK1 expression in ICH group and A+ICH group; enhance ERK2 expression in A+ICH group.*

Table 3.

ERK 1/2 expression in brain cortex after modeling intracerebral hemorrhage.

Group		ERK1	ERK2
1	Control	0,203±0,062	0,318±0,065
2	ICH	0,150±0,049	0,321±0,063
3	A+ICH	0,113±0,063	0,394±0,125
p-value		P_{1-2}=0,031 P_{1-3}=0,007 P_{2-3}=0,046	P_{1-2}=0,369 P_{1-3}=0,142 P_{2-3}=0,147

Note: values are expressed in Mean±SEM

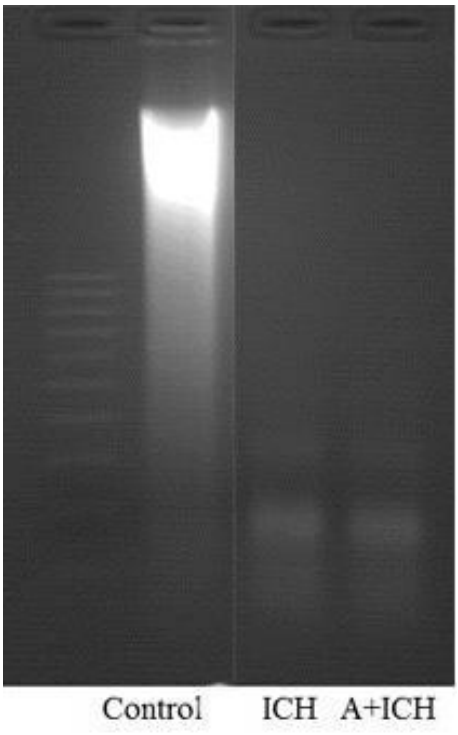

Figure 3. The DNA fragmentation in perifocal cortex after modeling intracerebral hemorrhage.
Note: necrotic type of DNA fragmentation, i.e. crushing to short nucleotide fragments.

REFERENCES

1. Andaluz N., Zuccarello M., Wagner K.R. Experimental animal models of intracerebral hemorrhage // Neurosurg. Clin. N. Am. – 2002. – Vol. 13. – P. 385–393.

2. Chevari S, Chaba I, Sekej J. Role of SOD in oxidative processes cells and its method of determination in biological material // Lab Delo. – 1985. – ¹ 11. – P. 678-681.

3. Chu K., Jeong S.W., Jung K.H., Han S.Y., Lee S.T., Kim M., Roh J.K. Celecoxib induces functional recovery after intracerebral hemorrhage with reduction of brain edema and perihematomal cell death // J. Cereb. Blood Flow Metab. – 2004. – Vol. 24. – P. 926– 933.

4. Hu Q., Vakhmjanin A., Li B., Tang J., Zhang J.H. Hyperbaric oxygen therapy fails to reduce hydrocephalus formation following subarachnoid hemorrhage in rats // Med. Gas. Res. – 2014. – Vol. 4. – P. 12.

5. Katsuki H. Animal models of intracerebral hemorrhage for development of new drug therapy // Nihon Yakurigaku. Zasshi. – 2014. – Vol. 144(3). – P. 120-125.

6. Koroljuk M.A., IvanovL.I., MayorovI.G., Tokarev V.E. Method for determination of catalase activity // Lab. business. – 1988. – ¹1. – P. 16-19.

7. Krushinski-´ A.L., Kuzenkov V.S., D'iakonova V.E., Reutov V.P. The influence of inhibitors of neuronal and inducible NOsynthases on experimental hemorrhagic stroke // Zh. Nevrol. Psikhiatr. Im. S.S. Korsakova. – 2014. – Vol. 114(8 Vypusk 2 Insul't). – P. 21-27.

8. MacLellan C.L., Auriat A., McGie S.C., Yan R.H., Huynh H.D., De Butte M.F., Colbourne F. Gauging recovery after hemorrhagic stroke in rats: implications

for cytoprotection studies // J. Cereb. Blood. Flow Metab. – 2006. – Vol. 26. – P. 1031–1042.

9. Makarenko A., Kositsin N., Pasikova N. et al. Model of local haemorrhage in different brain regions of experimental animals // J. of Higher Nervous Activity. – 2002. – Vol. 52(6). – P. 760–763.

10. Paxinos G. The rat brain in stereotaxic coodiates / G. Paxinos, C. Watson. – San Diego: Academic Press, 2008. – 400p.

11. Shiba M., Fujimoto M., Imanaka-Yoshida K., Yoshida T., Taki W., Suzuki H. Tenascin-C causes neuronal apoptosis after subarachnoid hemorrhage in rats // Transl. Stroke Res. – 2014. – Vol. 5(2). – P. 238-247.

12. Wagner K.R., Xi G., Hua Y., Kleinholz M., de Courten- Myers G.M., Myers R.E., Broderick J.P., Brott T.G. Lobar intracerebral hemorrhage model in pigs: rapid edema development in perihematomal white matter // Stroke. – 1996. – Vol. 27, ¹ 3. – P. 490-497.

УШКОДЖЕННЯ КОРИ ПІВКУЛЬ ВЕЛИКОГО МОЗКУ ЩУРІВ ПІСЛЯ ЕКСПЕРИМЕНТАЛЬНОГО МОДЕЛЮВАННЯ ІНТРАЦЕРЕБРАЛЬНОЇ ГЕМАТОМИ

Олійник Т.М. [1], Савосько С.І. [1], Бібікова В.М. [2]
[1] Національний медичний університет імені О. О. Богомольця, м. Київ, Україна
[2] Дніпропетровська медична академія, м. Дніпропетровськ, Україна

Резюме. В статті описано дослідження структурних та метаболічних змін кори мозку при геморагічному інсульті і впливі збільшеного артеріального тиску на перебіг цих порушень. Для досягнення мети проведені гістологічне дослідження, визначення рівня активності каталази і СОД, ERK кіназ і ступінь фрагментації ДНК.

Структурні та метаболічні зміни в корі головного мозку щурів були досліджені за різного ступеня геморагічного інсульту. Внутрішньомозковий крововилив, перифокальний набряк та ферментативна дисфункція збільшувалися при введенні адреналіну перед моделювання інсульту. Значну різницю встановлено в інших показниках в порівнянні з контрольною групою із легкою формою інсульту.

Рівень супероксиддисмутази в моторній корі був достовірно меншим на 23,6% з тенденцією до зниження у групі із моделювання інсульту після введення високих доз адреналіну. Рівень каталази відповідно зменшився на 15,4%. Ступінь фрагментації ДНК в перифокальній корі головного мозку суттєво не відрізнявся між двома моделями геморагічного інсульту і відповідав типу некрозу, а не апоптозу. Ці дані показують, що ішемічне ушкодження тканини головного мозку знижує антиоксидантний потенціал, що є фактором прогресування дегенеративних процесів перифокальної кори мозку.

Отримані результати вказують на розвиток значного окислювального стресу при геморагічному інсульті і його прогресуванні при високому артеріальному тиску. Ці дані можуть бути використані для дослідження патогенезу геморагічного інсульту і визначення дію нейропротекторів,

метаболічних і протинабрякових препаратів.

Ключові слова: інсульт, набряк мозку, артеріальний тиск, ферментативна дисфункція.

ПОВРЕЖДЕНИЕ КОРЫ БОЛЬШОГО МОЗГА КРЫС ПОСЛЕ ЭКСПЕРИМЕНТАЛЬНОГО МОДЕЛИРОВАНИЯ ИНТРАЦЕРЕБРАЛЬНОЙ ГЕМАТОМЫ

Олейник Т.Н. [1], *Савосько С.И.* [1], *Бибикова В.Н.* [2]
[1] *Национальний медицинский университет имени А. А. Богомольца, г. Киев, Украина*
[2] *Днепропетровская медицинская академия, г. Днепропетровск, Украина*

Резюме. В статье описано исследование структурных и метаболических изменений коры мозга при геморрагическом инсульте и влиянии увеличенного артериального давления на ход этих нарушений. Для достижения цели проведены гистологическое исследование, определение уровня активности каталазы и СОД, ERK киназ и степень фрагментации ДНК.

Структурные и метаболические изменения в коре головного мозга крыс были исследованы при разной степени геморрагического инсульта. Внутримозговое кровоизлияние, перифокальный отек и ферментативная дисфункция увеличивались при введении адреналина перед моделированием инсульта. Значительную разницу установлено в других показателях по сравнению с контрольной группой с легкой формой инсульта.

Уровень супероксиддисмутазы в моторной коре был достоверно меньше на 23,6% с тенденцией к снижению в группе моделирования инсульта после введения высоких доз адреналина. Уровень каталазы соответственно уменьшился на 15,4%. Степень фрагментации ДНК в перифокальной коре головного мозга существенно не отличался между двумя моделями геморрагического инсульта и соответствовал типа некроза, а не апоптоза. Эти данные показывают, что ишемическое повреждение ткани головного мозга снижает антиоксидантный потенциал, является фактором прогрессирования дегенеративных процессов перифокальной коры мозга.

Полученные результаты указывают на развитие значительного окислительного стресса при геморрагическом инсульте и его прогрессировании при высоком артериальном давлении. Эти данные могут быть использованы для исследования патогенеза геморрагического инсульта и определения действие нейропротекторов, метаболических и противоотечных препаратов.

Ключевые слова: инсульт, отек мозга, артериальное давление, ферментативная дисфункция.