RESEARCH RESULT
НАУЧНЫЙ РЕЗУЛЬТАТ
The development of hypertensive neuroretinopathy model on wistar rats / A.A. Peresypkina, A.A. Dolzhikov, V.O. Gubareva, E.A. Levkova, A.S. Shabelnikova // Research result: pharmacology and clinical pharmacology. – 2017. – Vol. 3, №1 – P. 18-31.

UDC: 57.084.1 **DOI: 10.18413/2500-235X-2017-3-1-18-31**

Peresypkina A.A.
Dolzhikov A.A.
Gubareva V.O.
Levkova E.A.
Shabelnikova A.S.

THE DEVELOPMENT OF HYPERTENSIVE NEURORETINOPATHY MODEL ON WISTAR RATS

Belgorod State University, 85 Pobedy St., Belgorod, 308015, Russia, e-mail: peresypkina_a@bsu.edu.ru

Abstract. A model of hypertensive neuroretinopathy was developed on Wistar line rats with administration of non-selective NO-synthase blocker N-nitro-L-arginine methyl ester (L-NAME) in a dose 12.5 mg/kg within 28 days on the background of single increased intraocular pressure (IOP) to 110 mmHg. As a result of the interim evaluation of pathology simulation mode the most optimal was a model of hypertensive neuroretinopathy with 5-minute increased IOP at day 26 of the experiment on the background of L-NAME for further study of neuroretinoprotective properties of pharmacological agents. Changes formation of the retina and optic nerve disc (OND), observed in the hypertensive neuroretinopathy, confirmed by ophthalmoscopy, laser Doppler flowmetry (LDF), electroretinography (ERG), histological studies in a group of animals with the pathology modeling at the 29 day of experiment.
Keywords: hypertensive neuroretinopathy, ophthalmoscopy, laser Doppler flowmetry, electroretinography.

Introduction.

Changes in the eye because of high blood pressure (BP) have a special place [1]. Hypertensive neuroretinopathy is a combination of signs of hypertensive retinopathy and changes in the optic disc (disc edema, increase in size, fuzzy edge, etc.). In the final stage, there may be an atrophy of the optic disc. Severe swelling of the optic disc – a sign of malignant hypertension stage.

The retina is the only organ where blood vessels state can be directly evaluated by providing important information about the risk of hypertension [2]. Angiopathy of the retina of hypertensive type is correlated with a high risk of cardio – vascular diseases in general, but not included in the risk assessment tools. Currently abroad the research of hypertensive retinopathy as determinant of target organ damage in hypertension are actively conducted [3, 4, 5].

Speaking about the pathogenesis of hypertensive retinopathy, it should be noted three main factors: the restriction and increased vascular permeability, and arteriosclerosis. The changes occuring in the fundus are typical of chronic, acute or malignant hypertensive retinopathy.

Against the background of hypertension the diameter of the arteries is reduced, cytoarchitectonics of bloodstream is disrupted. Due to vascular wall hypertrophy the vascular resistance is increased, their lumen is decreased which results in regional ischemia.

Acute optic neuropathy is characterized by swelling of the optic disk, hemorrhages on the retinal surface in the peripapillary area due to the narrowing of the lumen of peripapillary choroidal vessels supplying the optic disc. Its ischemia leads to disruption of axoplasmatic current, which proves that this defeat is nothing but a form of anterior ischemic optic neuropathy.

Patients with chronic hypertensive retinopathy do not show, as a rule, no complaints, they have no symptoms. Hypertensive retinopathy is detected by the presence of comorbidity: senile sclerosis of vessels and arterioles. The phase of vasoconstriction occurs due to diffuse spasm accompanied by increased blood pressure for some time, leading to an increase in retinal arteriolar tone. Sclerotic phase develops due to the increase in thickness or hypoplasia of the vascular wall, and hyaline degeneration, characterized by narrowing and arteriolar tortuosity [6].

All retinal changes occur due to high blood pressure, therefore primarily the treatment is directed to the hypertension therapy. Antihypertensives,

symptomatic treatment (angioprotectors, vasodilatators, retinoprotectors) are applied, which are nonspecific therapy and not always achieve the desired result. Therefore, effectiveness improving of pharmacological correction of hypertensive neuroretinopathy is an urgent task of the experimental and clinical pharmacology and ophthalmology.

In connection with the above, it should be noted the relevance of developing an adequate model of hypertensive neuroretinopathy for further study of neuroretinoprotective properties of pharmacological agents using instrumental and histological methods of analysis.

Objective: to develop an optimal model of hypertensive neuroretinopathy on Wistar rats to assess the neuroretinoprotective effects of pharmacological agents.

Materials and methods.

Experiments were carried out on male Wistar rats weighing 225-275 g. Rats were taken to study with no outward signs of disease, passed quarantine regime.

Operations and other manipulations were performed on rats under general anesthesia by intraperitoneal (i/p) introducing an aqueous solution of chloral hydrate 300 mg/kg rat weight.

Hypertensive neuroretinopathy simulation was performed by daily i/p L-NAME administration in a dose 12.5 mg/kg rat weight for 28 days [7] and the increase in IOP to 110 mmHg. [8] on day 26 of the experiment through the provision of mechanical pressure on the anterior chamber.

After anesthesia by the i/p introduction of chloral hydrate solution at 26 day of the experiment the animal was fixed in position on the side, followed by an increase in IOP.

To measure blood pressure in rats (tail) a system of non-invasive measurement of blood pressure for small animals NIBP200 was used in the complex Biopac-systems MP-150.

To investigate the fundus of experimental animals a direct ophthalmoscopy was used on 29 day of the experiment (ophthalmoscope Bx a Neitz, Japan). To expand the pupil the eye drops Irifrin 2.5% were used. Ophthalmoscope has been approached to the rat eye and we sent in it a beam of light from a distance of 0.5-2 cm to obtain a clear picture of the fundus image. In the dim image of the fundus we picked up the lens by turning the disc of ophthalmoscope, which gives crisp image details of the fundus. To zoom a lens Osher MaxField 78D model OI-78M has been used.

For subsequent statistical processing the degree of change, detected during ophthalmoscopy, were ranking (Tab. 1). [7].

Table 1

Methods of integrated semiquantitative evaluation of the fundus changes, detected during ophthalmoscopy (in grades)

A set of attributes of fundus changes	Grades
Optic disc is circular or oval shape and stands out from the fundus in pale – pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. From the middle of the optic nerve exit the central vessels of the retina. Blood vessels of the retina don`t have anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink.	0
Angiopathy. Symptom Salus-Hun I. It is characterized by the presence of sclerosis of retinal vessels in fundus and "phenomenon of chiasm", which occurs due to indentation of artery at the site of chiasm with extended vein. Expansions of vein are on both sides of the chiasm. Symptom Guist – expansion and corkscrew curl of venules located around the macular; observed in hypertensive disease.	1
Angiosclerosis. Symptom of copper wire – yellow glow of the retinal arteries; sign of hypertensive retinal angiopathy. Symptom Salus-Hun II – the formation of bulges in arteries and veins chiasm.	2
Symptom Salus-Hun III – the disappearance of the vein at the site of crossing due to the formation of the arcuate bend, sinking deep into the retinal tissue. Symptom of silver wire. Increased vascular permeability.	3
Retinopathy. "Cotton" exudates. Hemorrhages. In the macular area may be deposits of hard exudates in a star shape.	4
Hypertensive neuroretinopathy. Severe discoloration of the optic nerve disc. Swelling of the optic disc and peripapillary retina. Multiple foci of hemorrhage and "cotton" exudate, indicates the growing ischemia.	5

Measuring of the microcirculation level in the rats retina was performed by LDF after examining the fundus. Registration is carried out by means of hardware and software Biopac-systems MP-150 and the needle-type sensor TSD-144 (USA) with AcqKnowledge 4.2 program. After animal anesthesia assessment of microcirculation level was carried out in ten points on the circumference of the eyeball, the recording duration of the microcirculation level readings at one point was 20 seconds. From the microcirculation level results at every point the average value has been calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. Value of microcirculation in the animal group was calculated as the average of the values obtained from each experimental animal in group [9].

ERG was performed immediately after the registration of the microcirculation level. For this the animals were kept in the dark for 30 minutes [10], further the animals were anesthetized (chloral hydrate, 300 mg/kg, i/p) and fixed on the table, isolated from the electromagnetic radiation. Corneal silver electrode was placed on the cornea that has been soaked by saline solution for better contact, the reference needle electrode EL452 has been placed subcutaneously in the region of the skull, ground needle electrode EL450 has been placed subcutaneously in the base of the tail. Strobe flash of white light that is connected to the stimulator STM200 by company Biopac System, Inc. (USA) has been placed behind the back of the animal, ERG registration was carried out in response to a single stimulation. Evoked biopotentials were run at a frequency of 1-1000 Hz, amplified, averaged and presented graphically on the screen using the Biopac-systems MP-150 with a computer program AcqKnowledge 4.2 (USA). ERG-recording was carried out for 0.5 seconds in each rat in groups. To assess the degree of functional damage to the retina we evaluated the ratio of amplitudes of a- and b-wave of ERG – the coefficient b/a [11]. From ten values in each group were taken the average, which was added to the protocol.

For histological examination the eyes were removed after the ERG, on 29 day of experiment, fully with adjacent structures and fixed by immersion method in 10% formalin solution. After fixation, eyes were cut into two parts in the meridian direction substantially through the center and both halves embedded in paraffin according to standard procedures. Also, the serial sections in a meridian direction were made 5-7 mm thick, which were stained with hematoxylin and eosin. Histological processing steps performed using the «Leica» equipment company (Germany). For microscopic examination and archiving the finished micropreparations were scanned using a computer image archiving and analysis system "Mirax Desk". Image analysis and morphometry performed using «Pannoramic Viewer» program 1.15.4. In a strictly perpendicular direction the thickness of the retinal layers were measured with an accuracy of hundredths of a micrometer. 5 representative areas of each animal, free from processing artifacts, were measured. Quantitative data were recorded in the MS Excel spreadsheets.

For all data the descriptive statistics were used: data are checked for normal distribution. Distribution type was determined by using the criterion of Shapiro-Wilk. In case of normal distribution the average value (M) and standard error of the mean (m) were calculated. In cases of abnormal distribution the median (Me) and the quartile range (QR) were calculated.

Between-group differences were analyzed by parametric (t-Student criterion) or non-parametric (Mann-Whitney test) methods, depending on the type of distribution. Differences were determined at 0.05 significance level. Statistical analyzes were performed by using Statistica 10.0 software.

The main part.

We have proposed a modification of hypertensive neuroretinopathy model on Wistar line male rats, in which increased IOP is carried out by mechanical pressure (110 mm Hg) to the front chamber of the eye on 26 day of the experiment on the background of daily administration of L-NAME in a dose 12.5 mg/kg within 28 days.

At the heart of pathogenesis is the development of hypertension in rats to 29 day of the experiment (SBP 204.8 mmHg, DBP 164.2 mmHg in a group with pathology; SBP 139.2 mmHg, DBP 104.2 mmHg in the intact group, $p<0.05$).

The confirmation of the formation of hypertensive neuroretinopathy on 29 day of the experiment were the results of ophthalmoscopy, LDF, ERG and histological studies.

The evaluation of the interim regime of increased IOP was performed. To find the optimal time of IOP increase to 110 mmHg the experiment included 5 groups of animals:

the first (n = 10) – intact animals,

the second (n = 10) – with the introduction of L-NAME within 28 days,

the third (n = 10) – with the introduction of L-NAME within 28 days + increased IOP within 2 min,

the fourth (n = 10) – with the introduction of L-NAME within 28 days + increased IOP within 5 min,

the fifth (n = 10) – with the introduction of L-NAME within 28 days + increased IOP within 10 min.

After the IOP increase after 72 hours of reperfusion [8], on 29 day of the experiment, the ophthalmoscopy, measurement of the microcirculation in the retina by LDF, determination of the functional condition of the retina by ERG, enucleation of eyes for histological studies were performed.

Results.

In accordance with the study design, the duration of a single IOP increasing in hypertensive neuroretinopathy simulation with L-NAME administration was 2.5 and 10 min, followed by reperfusion period lasting 72 hours.

In accordance with the protocol, after the IOP increase after 72 h of reperfusion (29 day of experiment) animal anesthesia was performed (chloral hydrate solution i/p 300 mg/kg). Then ophthalmoscopy, assessment of the microcirculation

level in the retina, retinal electrophysiological state and taking the material for histological studies were performed.

Example of ophthalmoscopy on intact animal is shown in fig. 1.

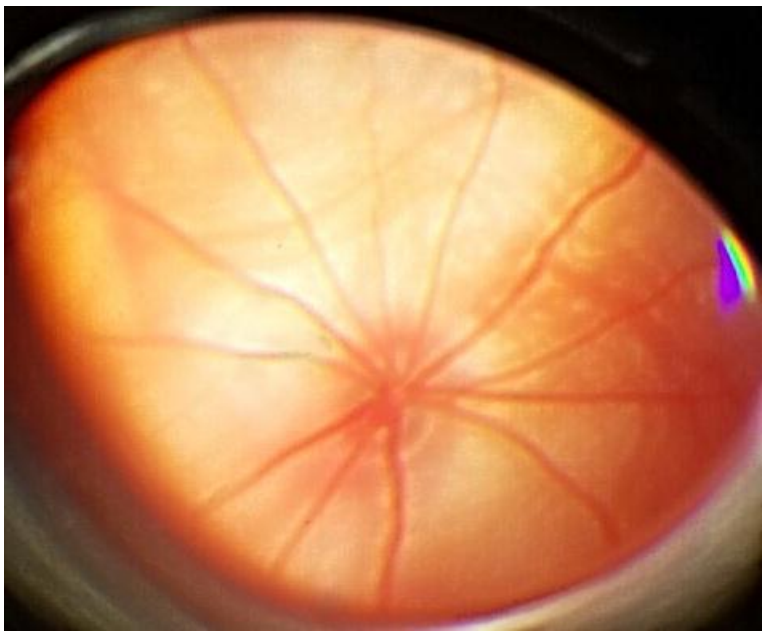

Figure 1. Example of ophthalmoscopy on intact Wistar rat. Optic disc is circular or oval shape and stands out from the fundus in pale – pink. The boundaries of the optic nerve disc are clear. It lies in the plane of the retina. From the middle of the optic nerve exit the central vessels of the retina. Blood vessels of the retina don`t have anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink

In the group with daily administration of L-NAME in a dose 12.5 mg/kg on day 29 of the experiment the retinal angiopathy of hypertensive type was observed. Ophthalmoscopic picture: optic disc is circular or oval shape and stands out from the fundus in pink. The boundaries of the optic nerve disc are clear. Veins are congested, full-blooded, crimped at the periphery. Arteries are narrowed, slightly crimped. Retina is palely (ischemic). Symptom Salus-Hun I.

Ophthalmoscopy example on rat in pathology modeling on the background of L-NAME administration and single IOP increase within 2 min is shown in fig. 2. We observed a pattern similar to retinopathy on the background of arterial hypertension without lesions of the optic disk.

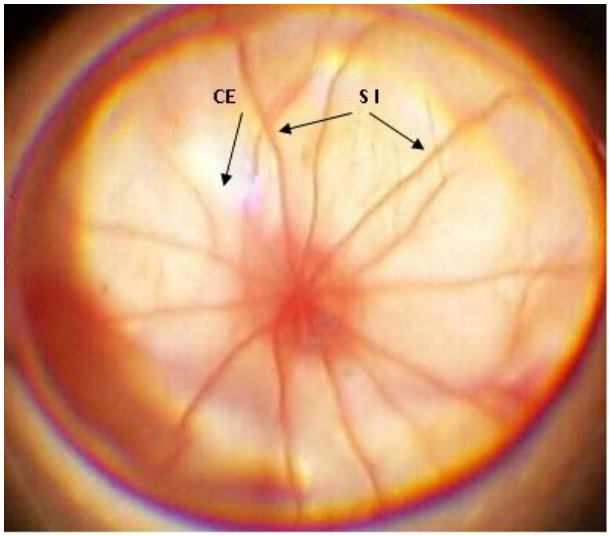

Figure 2. Example of ophthalmoscopy on Wistar rat with pathology simulation on the background of L-NAME and a single IOP increase within 2 min. Optic disc is round or oval shape and stands out from the fundus of the eye in pink. The boundaries of the OND are clear. It lies in the plane of the retina. There are pockets of "cotton" exudate (arrow + CE). Viens are congested. Arteries are narrowed. Vessel caliber is uneven. Retina is palely (ischemic). Symptom Salus-Hun I (arrow + S I)

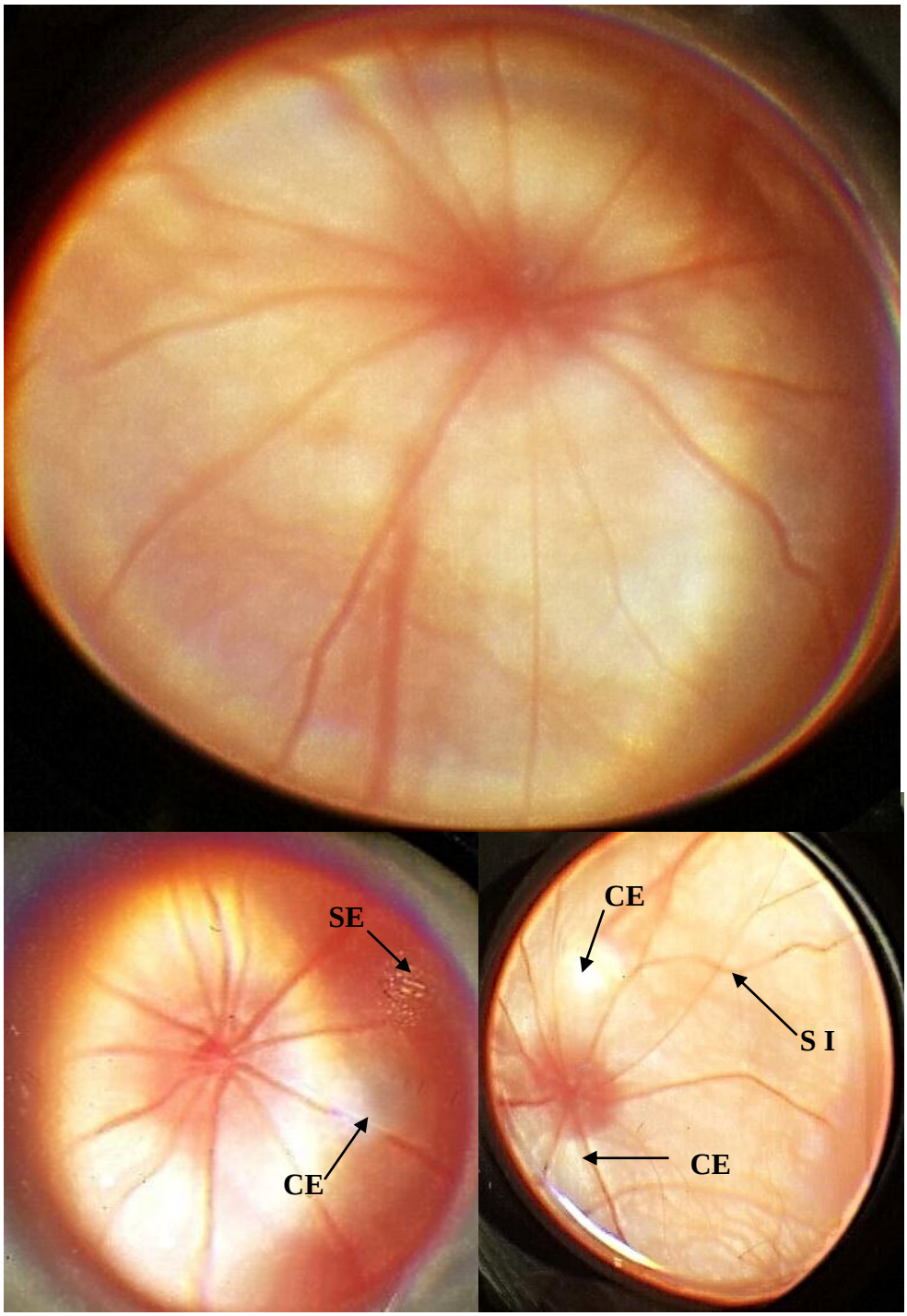

Figure 3. Examples of ophthalmoscopy on Wistar rats with pathology simulation on the background of L-NAME and a single IOP increase within 5 min. Optic disc is edematous, increased in size, edema extends to the retina. A slight blurring boundaries of OND. There are pockets of "cotton" exudate (arrow + CE), indicating the growing ischemia. Viens are congested, crimped at the periphery. Arteries are narrowed. Vessel caliber is uneven. Retina is palely (ischemic). Symptom Salus-Hun I (arrow + S I). In rare cases, the solid exudate deposits were observed (arrow + SE)

Examples of ophthalmoscopy on Wistar rats with pathology simulation on the background of L-NAME and a single IOP increase within 5 min are shown in fig. 3. We observed a pattern similar to hypertensive neuroretinopathy.

In the group with pathology simulation on the background of L-NAME and single IOP increase within 10 min irreversible changes, presumably, typical for neovascular glaucoma were observed (fig. 4).

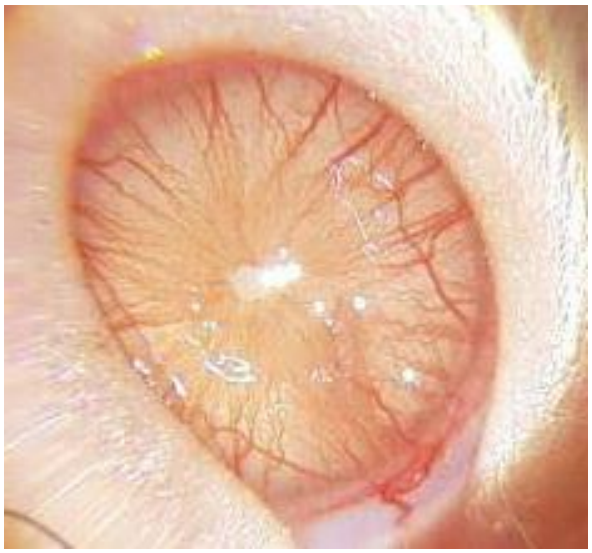

Figure 4. Pathological changes in the eye on the background of L-NAME administration and single IOP increase within 10 min

Thus, the results of fundus research during ophthalmoscopy on experimental animals have found that the optimal model of pathology was with single 5-minute IOP increase to 110 mmHg on day 26 of the experiment on the background of daily i/p L-NAME administration in a dose 12.5 mg/kg within 28 days for the study of the neuroretinoprotective properties of pharmacological agents.

Integral evaluation showed, respectively, 0 and 4-5 points for intact rats and rats with hypertensive neuroretinopathy (tab. 2).

Table 2

Integral assessment of fundus changes, detected during ophthalmoscopy (scores 0-5; n = 10)

Experimental groups	Integral assessment in scores
Intact	0
With L-NAME introduction, 12,5 mg/kg within 28 days	3*
With L-NAME introduction, 12,5 mg/kg within 28 days + IOP increase within 2 min	3-4*
With L-NAME introduction, 12,5 mg/kg within 28 days + IOP increase within 5 min	**4-5***
With L-NAME introduction, 12,5 mg/kg within 28 days + IOP increase within 10 min	>5*

Note. * – p<0.05 compared with the group of intact animals

After modeling of hypertensive neuroretinopathy on rats on 29 day of the experiment we observed the deterioration of retinal blood flow, which is associated with the development of the pathological cascade of morphological and functional changes in the retina.

The level of the retinal microcirculation of intact animals was 743.0 ± 20.9 p.u. In the group with L-NAME administration after disease modeling on 29 day microcirculation level was decreased to 431,4 ± 13,8 p.u., which was significantly different from the values of intact animals (p<0.05). In the group with L-NAME administration within 28 days and an IOP increase within 2 min this indicator was 420,7 ± 10,5 p.u. (p<0.05 compared with the group of intact animals). In the group with L-NAME administration within 28 days and an IOP increase within 5 min microcirculatory level was 417.2 ± 13.1 p.u. (p<0.05 compared with the group of intact animals), which is less than the value in the group of intact animals by 44% and confirms the formation of retinal ischemia in the hypertensive neuroretinopathy simulation. In the group with L-NAME administration and an IOP increase within 10 min the rate was 804.1 ± 15.0 p.u. (p<0.05 compared with the group of intact animals). The increase in regional blood flow is attributed to compensatory pathological neoangiogenesis and later development of neovascular glaucoma.

Based on these results, it should be noted that the duration of ischemic episode with an IOP increase on the background of L-NAME administration significantly affects to the level of retinal microcirculation after 72 h of reperfusion.

Violations of local hemodynamics and formation of pathological changes in the optic disk led to changes in retinal electrophysiological state. To assess the severity of the functional changes in the retina we used the ratio b-wave amplitude to the amplitude of a wave of the ERG – the coefficient b/a [11]. After the measuring of the microcirculation level in the retina the ERG on evoked potential was performed. The data obtained are presented in tab. 3.

Table 3

The results of evaluation of retinal electrophysiological state on day 29 of the experiment (M ± m; n = 10), r.u.

Experimental groups	b/a
Intact	2.6±0.07
With L-NAME introduction, 12.5 mg/kg within 28 days	2.2±0.09*
With L-NAME introduction, 12.5 mg/kg within 28 days + IOP increase within 2 min	2.1±0.06*
With L-NAME introduction, 12.5 mg/kg within 28 days + IOP increase within 5 min	**1.9±0.08***
With L-NAME introduction, 12.5 mg/kg within 28 days + IOP increase within 10 min	1.1±0.05*

Note. * – p<0.05 compared with the group of intact animals

During the ERG found that the ratio b/a in the group of intact animals was 2.6 ± 0.07 r.u. After pathology modeling, on 29 day of the experiment, in group with L-NAME administration the ratio b/a was 2.2 ± 0.09 r.u., which was significantly different from the values of intact animals (p<0.05) and smaller than value in group of intact animals by 15%. In the group with L-NAME administration within 28 days and an IOP increase within 2 min the rate was 2.1 ± 0.06 r.u. (p<0.05 compared with the group of intact animals), which is less than in the group of intact animals by 19%. In the group with administration of L-NAME within 28 days and an IOP increase within 5 minutes b/a was 1.9 ± 0.08 r.u. (p<0.05 compared with the group of intact animals), which is less than the value of intact animals by 27%. In the group with administration of L-NAME and an IOP increase within 10 min the rate was 1.1 ± 0.05 r.u. (p<0.05 compared with the group of intact animals), which is less than in the group of intact animals by 58%.

Based on these data, we can conclude that the modeling of pathology causes a disturbance of the electrophysiological state of inner retinal layers due to violations of the retinal blood flow and the formation of chronic ischemia.

Data obtained during ophthalmoscopy, an integrated evaluation of the fundus changes, LDF and ERG in experimental groups, lead to the conclusion that the most optimal is the pathology model (of hypertensive neuroretinopathy) on the background of introduction of non-selective blocker of NO-synthase L-NAME in a dose of 12.5 mg/kg within 28 days and a single elevated IOP to 110 mmHg within 5 min on 26 day of the experiment for further research of neuroretinoprotective properties of pharmacological agents.

Based on the foregoing, the rats retina of the group with the introduction of L-NAME in a dose of 12.5 mg/kg within 28 days and an IOP increase within 5 min were subject to further histological examination. Histological examination revealed significant qualitative changes in all layers of the retina. The overall structure of the layers is loosened. During histological processing there were easily detached retina from the choroid, and the appearance of slit-like defects between the layers, simulating the swelling. In the photoreceptor layer the outer segments of rods and cones are less compact than normal, among them are found small slit and spheroid intervals (fig. 5 B, 6 A).

In the outer nuclear layer the dense arrangement of nuclei of photoreceptor cells, characteristic of norm, is lost, which indicates the decompaction of perikaryons location of photoreceptor cells (fig. 5 B, 6). Glial outer limiting membrane in normal tissue specimens is not detected (fig.6 A). Changes in the blood vessels of the microvasculature are also identified in the form of capillary stasis and formation of hyaline thrombus in small vessels (Fig. 6 B).

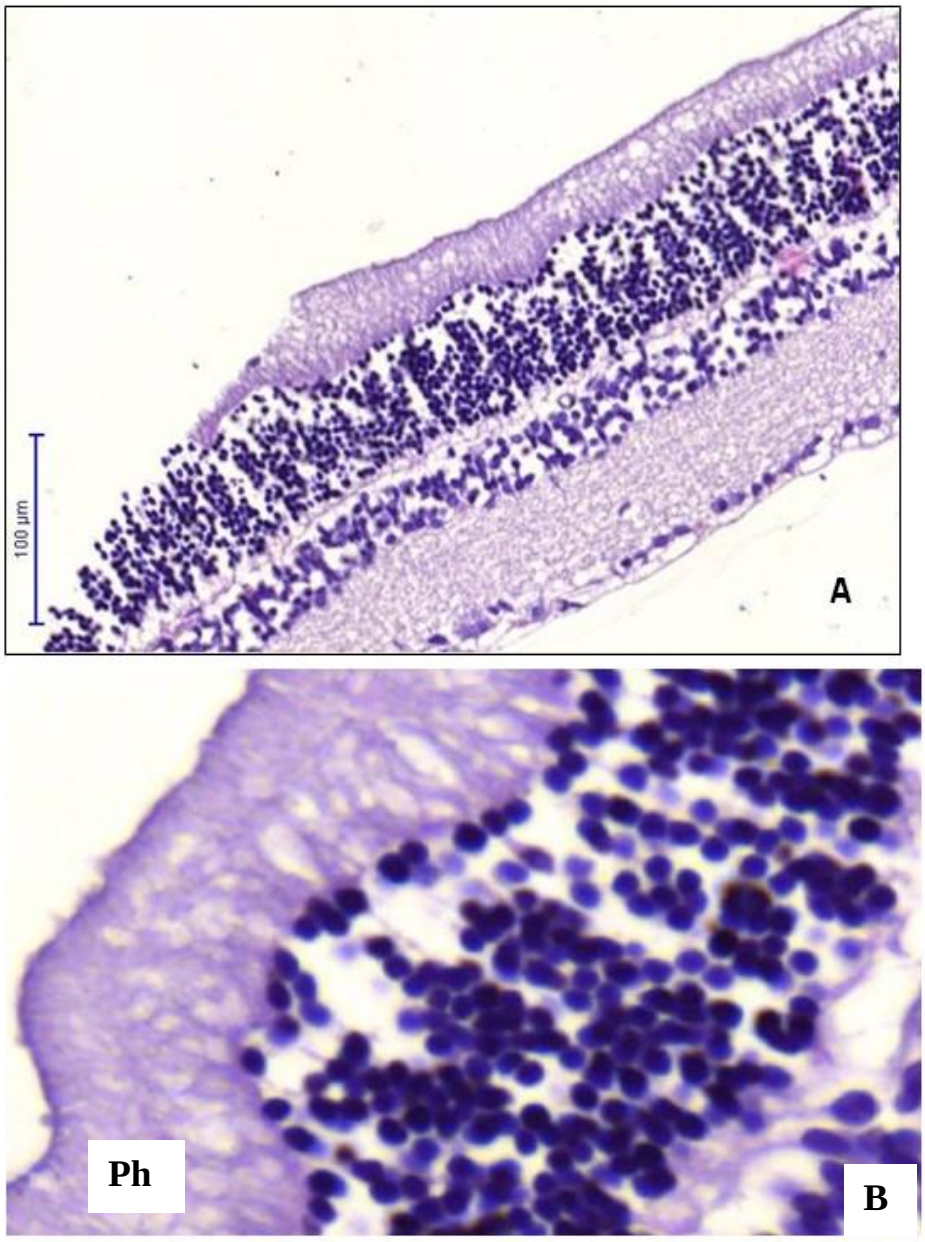

Figure 5. Histology of the retina in the group with experimental hypertensive neuroretinopathy: loosening of the layers structure (A), decompaction of location of the outer segments of rods and cones in the photoreceptor layer (Ph), decompaction of perikaryons location of photoreceptor cells in the outer nuclear layer (B)
Hematoxylin and eosin. Microphoto. x100 (A), x200 (B)

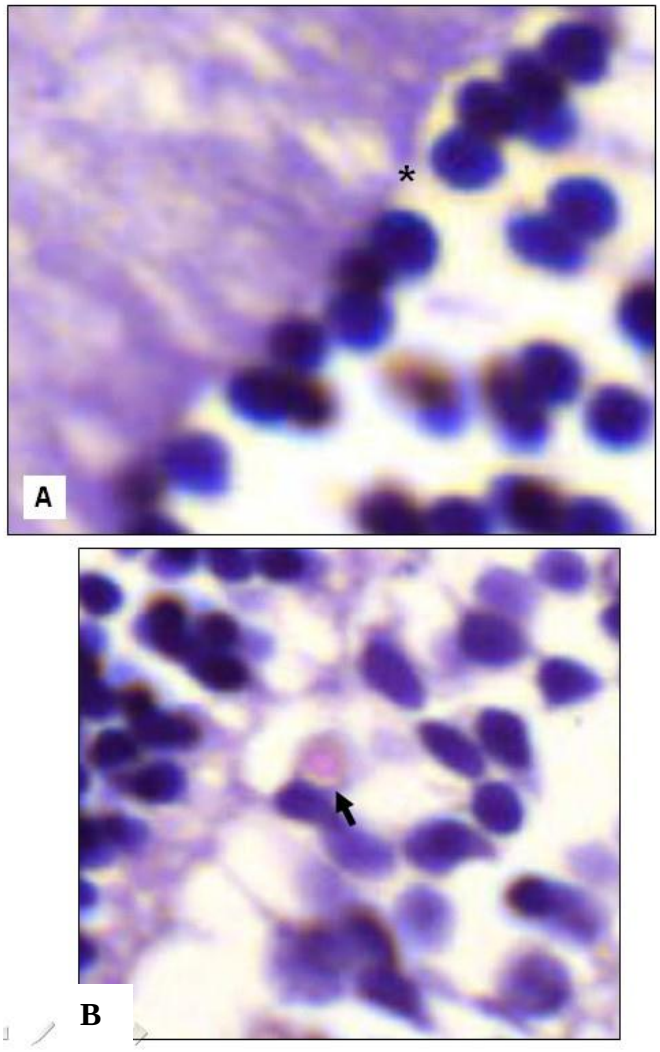

Figure 6. Changes in retinal layers in the group with experimental hypertensive neuroretinopathy:
A – structure decompaction of photoreceptor layer, spheroid and slit-like spaces between the outer segments of the rods and cones, the disorganization of the outer glial limiting membrane (its location indicated by an asterisk),
B – loose structure of the outer nuclear layer, hyaline thrombus in the small blood vessel is indicated by the arrow
Hematoxylin and eosin. Microphoto. x400

Until heavy changes are identified in the inner retinal layers. The inner nuclear layer is loosened, slotted bundles (fig. 7 A). The inner retinal layer, as well as outer, has loosened spongy appearance, which may reflect the relationship disruption of rames of ganglion and associative retinal neurons. Ganglionic layer neurons are exposed to severe changes in a hydropic dystrophy, chromatolysis, nuclear pinosis (fig 7, fig. 10), cytolytic changes are detected (fig. 8 B, fig. 11). The inner layers that formed by retinal neuronal processes (inner retinal layer and the nerve fibers) have spongy form (fig. 7, 8, 10), which may be indicative of axonal damage and disrupt of synaptic connections.

Described neuronal damages are occurring on the background of expressed microcirculatory disorders with severe venous congestion (fig. 7-9), arterioles damage with plasma impregnation of their walls (fig. 8 A).

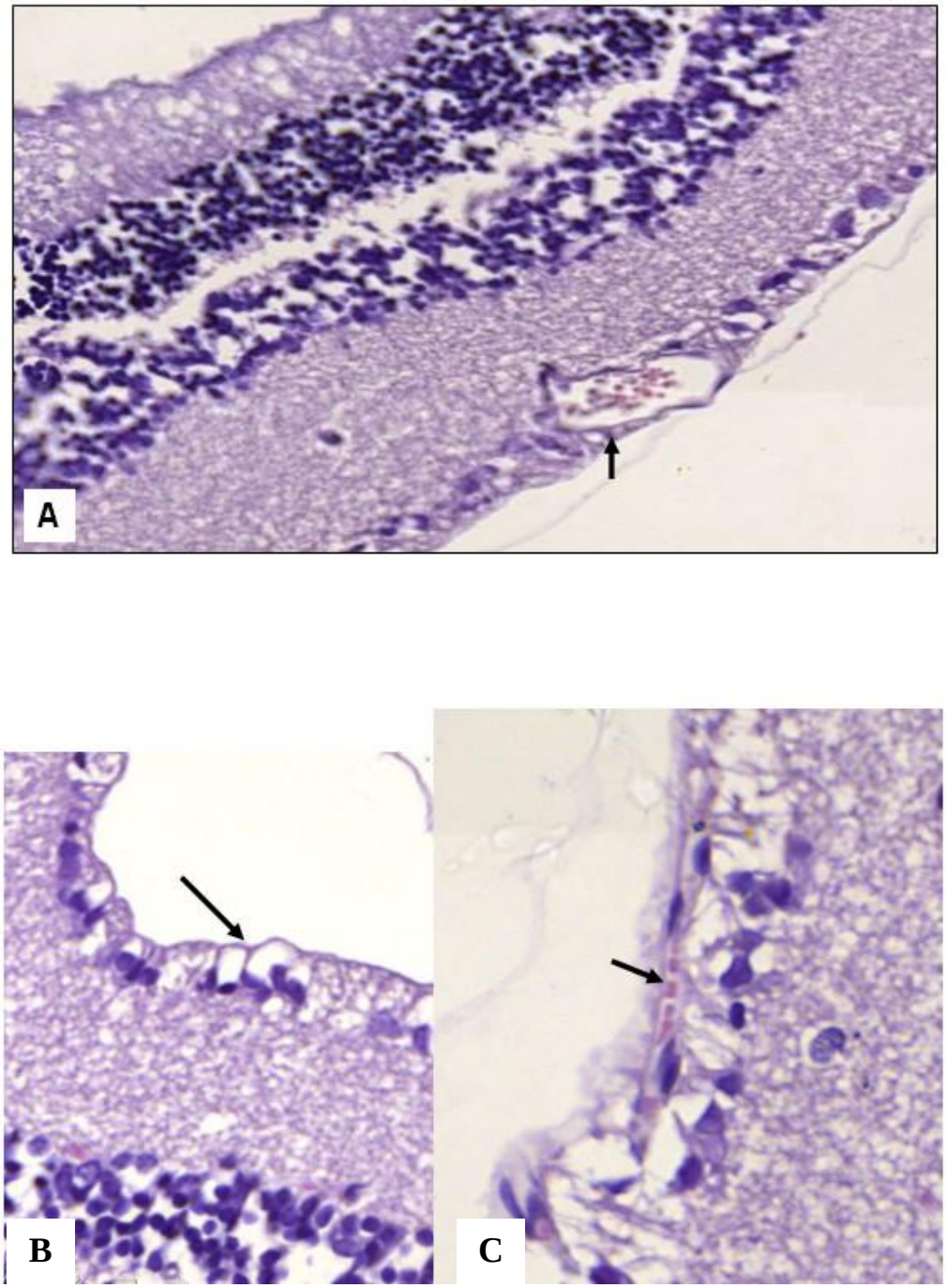

Figure 7. Changes in retinal layers in the group with experimental hypertensive neuroretinopathy: A – loose structure. The bundles in the inner nuclear layer, B – loosened structure of the inner retinal layer, expressed pericellular swelling in the ganglion layer (arrow), C – hydropic degeneration of ganglion neurons, capillary stasis with red blood cells in the form of "monetary pillar", the layer of nerve fibers (right part of microphoto) with spongiform changes. Hematoxylin and eosin. Microphoto. x200

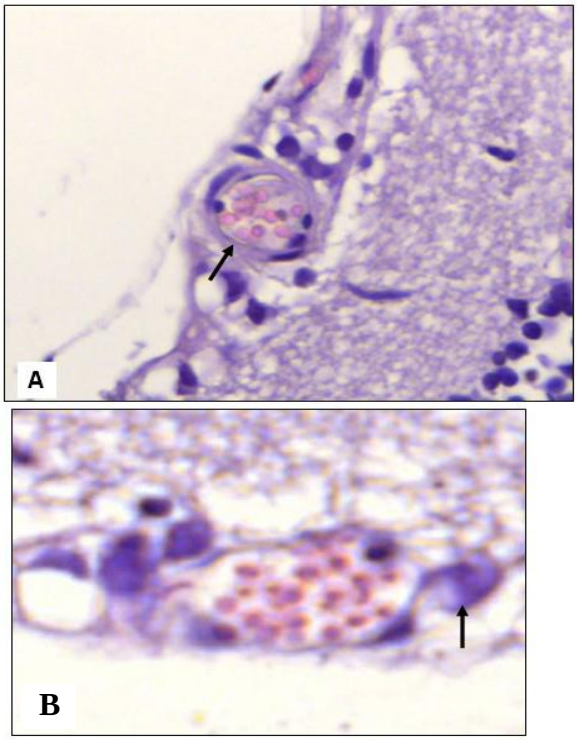

Figure 8. Vessels of retinal microcirculatory bed in the experimental group with hypertensive neuroretinopathy: A – plasmatic impregnation phenomenon in the wall of arterioles, B – full-blooded small vein, neuron fragments of ganglion layer with signs of cytolysis (arrow). Hematoxylin and eosin. Microphoto. x200

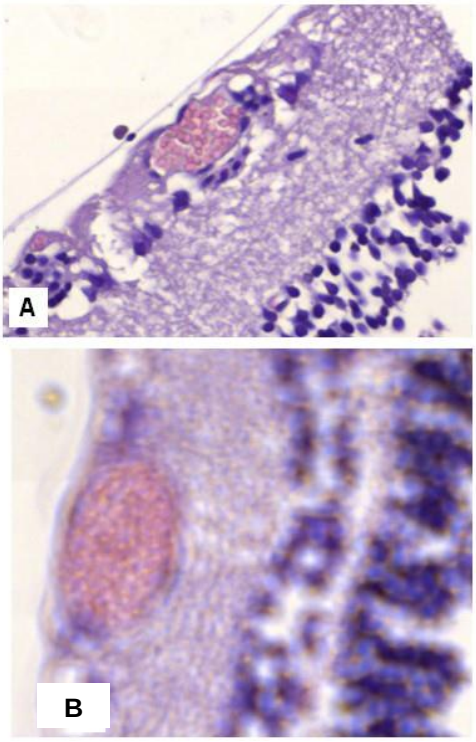

Figure 9. Severe venous congestion in the inner layers of the retina in the group with experimental hypertensive neuroretinopathy.
Hematoxylin and eosin. Microphoto. x100

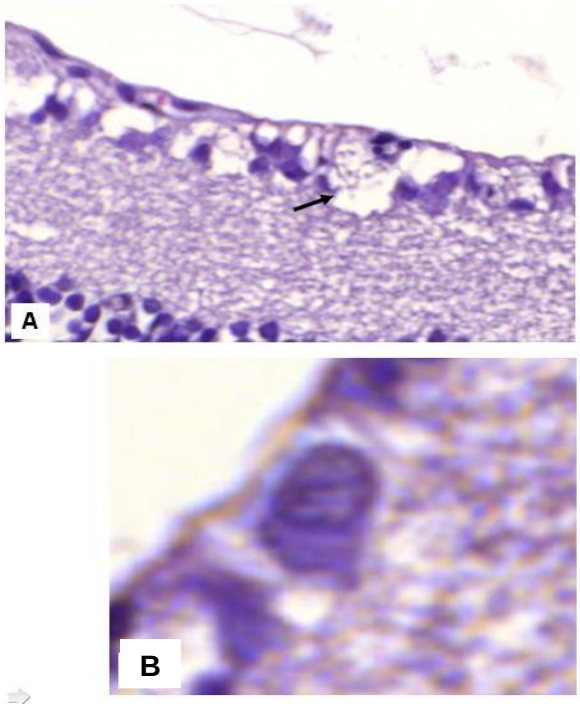

Figure 10. Changes in the ganglion layer and retinal nerve fiber layer in the group with experimental hypertensive neuroretinopathy:
A – hydropic dystrophy, cytolytic changes of ganglion neurons, B – chromatolysis, karyopyknosis in the ganglion neurons, spongiform changes in the nerve fiber layer. Hematoxylin and eosin. Microphoto. x200

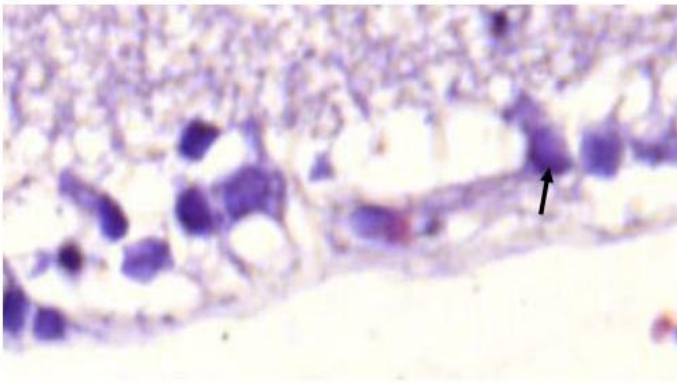

Figure 11. Heavy neuronal changes in the retinal ganglion layer in the group with experimental hypertensive neuroretinopathy: hydropic degeneration of neurons up to the balloon and total cytolysis, the arrow – a neuron with a total chromatolysis and karyopyknosis. Hematoxylin and eosin. Microphoto. x200

As a result, the proposed model of hypertensive neuroretinopathy is characterized by:

- severe vascular changes of hypertensive type in the retina, attributes of ischemic injury and pathological changes of the optic disc (4-5 points, $p<0.05$ compared with the group of intact animals) during ophthalmoscopy and integral evaluation of the fundus changes;
- statistically significant difference between values of the microcirculation level in the retina of rats with pathology from values in the intact group on day 29 of the experiment;
- significant reduction of coefficient b/a of ERG after the pathology simulation on 29 day of the experiment in comparison with the value in the group of intact animals.
- severe pathological changes of the inner layers of the retina revealed by histological examination.

Discussion.

Cardiovascular diseases are one of the main causes of death, and hypertension – the most common risk factor. Since the blood circulation in the retina are anatomically and physiologically similar to coronary and cerebral circulation, ophthalmologists received a "window" for the study of microcirculation. Idiopathic (primary) and malignant hypertension can become a cause of retinopathy. The etiology of idiopathic hypertension is not known, it is diagnosed when blood pressure levels in the arteries during systole >140 mmHg and diastolic >90 mmHg. Malignant hypertension is much rarer, it is diagnosed in 1% of patients with hypertension.

Hypertensive neuroretinopathy is more likely to develop in the late period of hypertensive disease and usually is a poor prognostic sign. It is characterized not only by changes in the blood vessels and retinal tissue, and involvement in the process of the optic disk, which becomes swollen, increased in size, swelling extends to the retina. Ophthalmoscopic picture is similar to the symptoms of stagnant disc, but unlike it marked a dramatic violation of color vision, decreased visual function: the decline of central vision and the narrowing of the field of view. At the end of neuroretinopathy an atrophy of the optic nerve may develop.

In malignant hypertension sclerotic processes are developing very quickly. Changes occuring in the fundus are typical for hypertensive retinopathy, choriopathy and optic neuropathy. Transudate deposition in the retinal layers of white oval due to the deposition of macromolecules during the arterioles. Moreover, there is the presence of structures in the form of "cotton lumps" and microaneurysms of capillaries obliteration zone.

Optic neuropathy is characterized by swelling of the optic disk, hemorrhages on the retinal surface in the peripapillary area due to the narrowing of the lumen of peripapillary choroidal vessels supplying the optic disc. Its ischemia leads to disruption of axoplasmatic current, which proves that this defeat is nothing but a form of anterior ischemic optic neuropathy [6].

The main factors in the development of retinal angiopathy of hypertensive type are disorders of common hemodynamics, local changes in the vessel walls. From local changes are the most important violations of the vascular endothelium [12].

The search of new methods of neuroretinoprotection for possible reduction of the damaging effect of ischemia, formed in hypertensive neuroretinopathy is an urgent task of pharmacology and ophthalmology. Segment of drugs for the treatment of vascular diseases of the eye as complications of hypertension is expedient to expand due to an increase in morbidity and lack of funds for targeted correction of ischemic lesions of the eye vessels [13].

Based on the fact that electrophysiological studies often have a decisive importance in the early and differential diagnosis of retinal disorders [14], to study the correction of functional changes in the retina, researcher must conduct a comprehensive analysis, including ophthalmoscopic, electroretinography, microcirculation research. Analysis of the dynamics of retinal electrogenesis allows to evaluate the nature and topography of retinal disorders, as well as to identify the most labile hypoxic retinal structure, their reaction to the correction by the medications.

The foregoing predetermined the need to develop and systematize the methodological

approaches to the assessment of the functional state of the retina and the subsequent optimization of correction of hypertensive neuroretinopathy. The studies conducted in our experiments on Wistar rats had developed a set of methodological approaches to assess the functional status of the retina, including instrumental methods of analysis (ophthalmoscopy, LDF, ERG) and histological studies of retinal layers.

The first step in exploring the possibility of correction of hypertensive neuroretinopathy was the development of its models on the laboratory Wistar rats. To do this, ophthalmoscopy, integrated semi-quantitative assessment of fundus changes are performed, the level of the retinal microcirculation and its electrophysiological state are evaluated, then the histological studies of retinal layers were held.

As a result of temporary mode evaluation of pathology simulation the most optimal was a model of hypertensive neuroretinopathy with administration of nonselective NO-synthase inhibitor L-NAME in a dose 12.5 mg/kg within 28 days and a single IOP increase up to 110 mmHg within 5 min on 26 day of experiment for creation of ischemia episode, followed by a reperfusion lasting for 72 hours.

It should be noted that the proposed method complex of functional changes associated with the development of hypertensive neuroretinopathy allows to evaluate sufficiently objectively the neuroretinoprotective effects of pharmacological agents.

Conclusion.

The study developed a model of hypertensive neuroretinopathy on rats of Wistar line, integrated semi-quantitative assessment of the fundus were held, the level of microcirculation, retinal electrophysiological status, histology retinal layers were assessed, that allowing to fully appreciate the formation of the changes in the modeling of pathology.

This model makes possible to evaluate sufficiently objectively the retinotropic effects of pharmacological agents.

Thus, the prospects become apparent to optimize the pharmacotherapy of conditions accompanied by retinal ischemia, which are closely linked with the task of forming the methodology of the study of antiischemic activity of pharmacologic agents based on an adequate assessment of the functional condition of the retina by instrumental methods of histological studies.

References

1. Wong TY, Mitchell P. Hypertensive retinopathy // *N Engl J Med*. – 2004. – Vol. 351. – P. 2310-2317. [Fulltext]

2. Konstantinidis L, Guex-Crosier Y. Hypertension and the eye // *Curr Opin Ophthalmol*. – 2016. – Vol. 27, №6. – P. 514-521. [Abstract]

3. Kolman SA, van Sijl AM, van der Sluijs FA, van de Ree MA. Consideration of hypertensive retinopathy as an important end-organ damage in patients with hypertension // *J Hum Hypertens.* – 2017. – Vol. 31, №2. – P. 121-125. doi: 10.1038/jhh.2016.49 [PubMed]

4. Cuspidi C, Meani S, Valerio C, et al. Prevalence and correlates of advanced retinopathy in a large selected hypertensive population. The Evaluation of Target Organ Damage in Hypertension (ETODH) study // *Blood Press*. – 2005. – Vol. 14, №1. – P. 25-31. [PubMed]

5. Cuspidi C, Macca G, Salerno M, et al. Evaluation of target organ damage in arterial hypertension: which role for qualitative funduscopic examination? // *Ital Heart J*. – 2001. – Vol. 2, №9. – P. 702-706. [PubMed]

6. A.R. Khurana, B. Khurana, S. Chauhan, et al. Hypertensive retinopathy an overview // *Haryana J. Ophthalmol*. – 2014.– Vol. VII.– P. 64-66. [Fulltext]

7. Peresypkina A.A., Gubareva V.O., Levkova E.A., Shabelnikova A.S. Correction of retinal angiopathy of hypertensive type by minoxidil, sildenafil in experiment // *Research result: pharmacology and clinical pharmacology*. – 2016. – Vol. 2, №4. – P. 34-44. doi: 10.18413/2500-235X-2016-2-4-34-44. [Fulltext]

8. Shabelnikova A.S. Correction of ischemic damage to the retina on application of pharmacological preconditioning of recombinant erythropoietin / A.S. Shabelnikova // *Research result: pharmacology and clinical pharmacology*. – 2016. – Vol.2, №2. – P. 67-90. [Full text]

9. Electrophysiological and histomorphometric changes in the retina of rats with pharmacological preconditioning by erythropoietin, by nikorandil on ischemia-reperfusion model of the eye [Text] / A.A. Peresypkina, M.V. Pokrovsky, A.S. Shabelnikova [et al.] // *Scientific statements BSU . Series: Medicine. Pharmacy*. – 2014. – Vol. 26-1, №11-1 (182). – P. 113-117. [eLIBRARY]

10. Effects of the duration of dark adaptation on the retinal function of normal SD rats [Text] / L. Zahng, Y.-h. Gu, J. An [et al.] // *Chinese journal of optometry ophthalmology and visual science*. – 2013. – Vol. 15, № 6. – P. 323-326. [Abstract]

11. Konstantinova, T.S. Tread and neurotoxic role of nitric oxide in the models of visual pathologies [Text]: Author. Dis. ... cand. biol. sciences: 03.00.02 / T.S. Konstantinova. – Moscow, 2009. – 26 p. [Full text]

12. Tinjust, D. Neuroretinal function during mild systemic hypoxia [Text] / D. Tinjust, H. Kergoat, J. V. Lovasik // *Aviat. Space Environ. Med.* – 2002. – Vol. 73, №12. – P. 1189-1194. [PubMed]

13. Analysis of the Russian wholesale market of eye drops for 2006 – 1-3 sq. 2012. [Text] / AA Arustamova, MV Pokrovskiy, AS Shabelnikova [et al.] // *Russian medical and biological messenger them. Acad. Pavlov*. – 2013. – № 4. – P. 71-76. [Full text]

14. Inhibition of synthesis of calpain by inhibitor E-64d in the retina subjected to ischemia/reperfusion [Text] / Zhiqing Chen, Ke Yao, Wen Xu [et al.] // *Molecular Biology*. – 2008. – Vol. 42, № 2. – P. 258-264. [Full text]

Peresypkina Anna Alexandrovna – PhD in Biological Sciences, Senior Lecturer of the Department of Pharmacology.

Dolzhikov Alexander Anatol`evich – MD, Professor of the Department of Histology.

Gubareva Viktoriya Olegovna – Graduate student of the Department of Pharmacology.

Levkova Elena Aleksandrovna – Graduate student of the Department of Pharmacology.

Shabelnikova Anna Sergeevna – Assistant Lecturer of the Department of Pharmacology.