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THE DEVELOPMENT OF HYPERTENSIVE NEURORETINOPATHY MODEL

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Abstract:

Introduction: *It should be noted the relevance of developing an adequate model of hypertensive neuroretinopathy for further study of neuroretinoprotective properties of pharmacological agents.*

Research tasks: *Development of the methodical approaches for evaluation of the neuroretino protective activity of pharmacological agents to objectively assess the degree of the protective activity of new and commonly used neuroretinoprotectors in ophalmology.*

Methods: *To investigate the fundus of experimental animals a direct ophthalmoscopy was used on 29th day of the experiment. The eye drops Irifrin 2.5% were used to expand the pupil. To zoom a lens Osher MaxField 78D model OI-78M has been used. Electroretinography was performed at once after the ophthalmoscopy. To assess the degree of functional damage to the retina we evaluated the ratio of amplitudes of a- and b- waves - the coefficient b/a. For all data, the descriptive statistics were used, and data are checked for normal distribution. Distribution type was determined by using the criterion of Shapiro-Wilk. 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.*

Results: *The results of ophthalmoscopy have found that the optimal model of pathology was with single 5-minute intraocular pressure (IOP) increase to 110 mmHg on day 26 of the experiment on the background of daily intraperitoneal N-nitro-L-arginine methyl ester (L-NAME) administration in a dose 12.5 mg/kg within 28 days: «Optic disc is edematous, increased in size, edema extends to the retina. Slight blurring boundaries of optic disc. There are pockets of "cotton" exudate, indicating the growing ischemia. Veins are congested, crimped at the periphery. Arteries are narrowed. Vessel caliber is uneven. Retina is palely (ischemic). Symptom Salus-Hun I. In rare cases, the solid exudate deposits were observed». In this group ratio 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%.*

Conclusion: *The study developed a model of hypertensive neuroretinopathy on rats of Wistar line, ophthalmoscopic picture and retinal electrophysiological status were assessed that allows to fully appreciate the formation of the changes in the modeling of pathology. This model makes possible to evaluate the retinotropic effects of pharmacological agents sufficiently objectively.*

Key words: *hypertensive neuroretinopathy, ophthalmoscopy, electroretinography.*

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INTRODUCTION:

Changes in the eye because of high blood pressure 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.). Speaking about the pathogenesis of hypertensive retinopathy, it should be noted three main factors: the restriction and increased vascular permeability, and arteriosclerosis. The changes occurring in the fundus are typical of chronic, acute or malignant hypertensive retinopathy [2].

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 [3].

All retinal changes occur due to high blood pressure; therefore, the treatment is primarily directed to the hypertension therapy. Antihypertensive, symptomatic treatment (angioprotectors, vasodilatators, retinoprotectors) is applied, which is a type of nonspecific therapy and does not always achieve the desired result. Hence, improvement of the effectiveness of pharmacological correction of hypertensive neuroretinopathy is an urgent task of the experimental and clinical pharmacology and ophthalmology. Moreover, study of pharmacological agents should be carried out on pharmacological targets [4, 5], in vivo models [6, 7].

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.

MATERIALS AND METHODS:

Experiments were carried out on male Wistar rats weighing 225-275 g. 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 [8] and the increase in IOP to 110 mmHg. [9] 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 29th day of the experiment (ophthalmoscope Bx a Neitz, Japan). The eye drops Irifrin 2.5% were used to expand the pupil. Ophthalmoscope has been introduced to examine 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 [10].

Electroretinography (ERG) was performed at once after the ophthalmoscopy. For this the animals were kept in the dark for 30 minutes [11], 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, and 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 [12, 13]. From ten values in each group the average was taken, which was added to the protocol.

For all data, the descriptive statistics were used, and 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 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 [9], on 29th day of the experiment, the ophthalmoscopy, determination of the functional condition of the retina by ERG were performed.

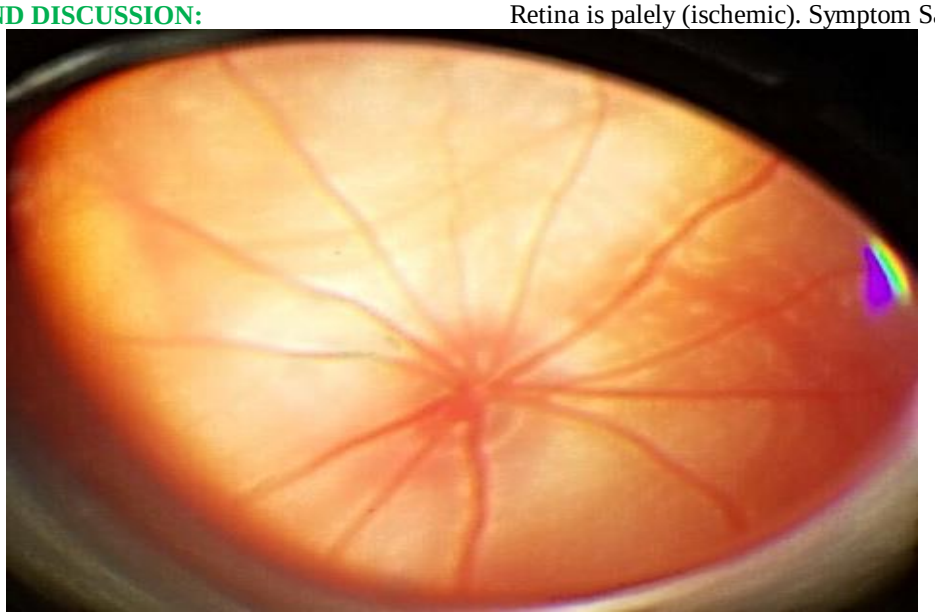
RESULTS AND DISCUSSION:

At the heart of pathogenesis of hypertensive neuroretinopathy is the development of hypertension in rats to 29th 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, $p<0.05$).

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 h.

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

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.



Fi 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 exits 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.

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.

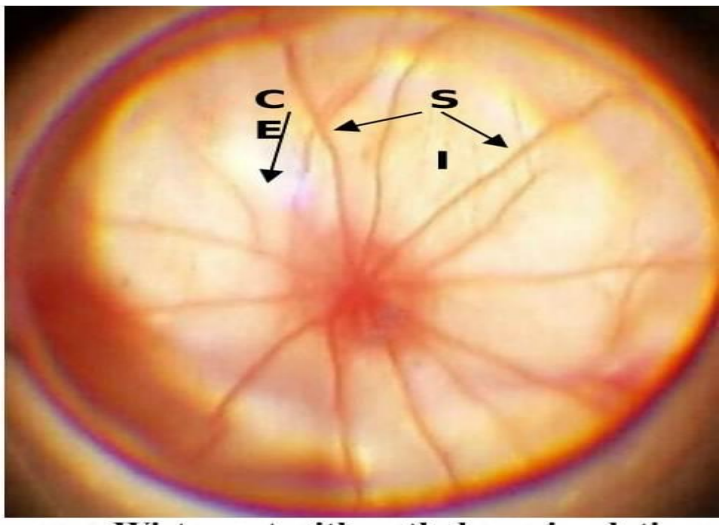
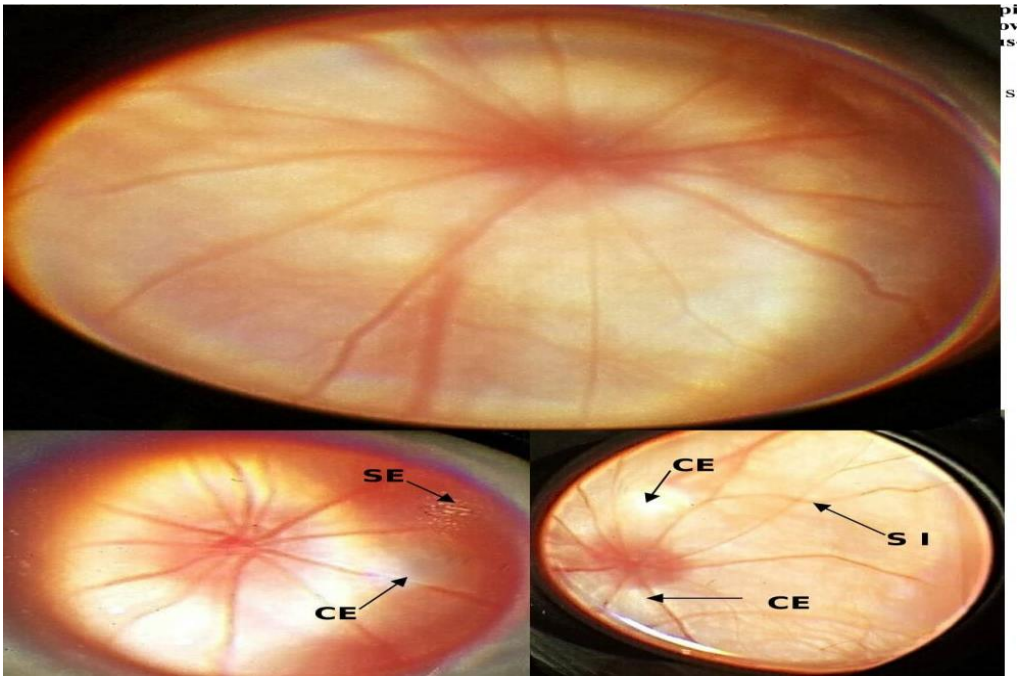


Fig 2: Example of ophthalmoscopy on Wistar rat with pathology simulation on the background of L-NAME and a single IOP increase within 2 min. Optic disc is round or oval shape and stands out from the fundus of the eye in pink. The boundaries of the OND are clear. It lies in the plane of the retina. There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



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.

Fig 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. Veins 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).

Table 1: 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*

Comment: * - p<0.05 compared with the group of intact animals.

In the group with pathology simulation on the background of L-NAME and single IOP increase irreversible changes, presumably, typical for neovascular glaucoma were observed 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.

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. The data obtained are presented in tab. 1.

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 29th 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%.

CONCLUSION:

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 and ERG in experimental groups, lead to the conclusion that the most optimal is the pathology model on the background of introduction of non-selective blocker of NO-synthase L-NAME in a dose 12.5 mg/kg within 28 days, and a single elevated IOP to 110 mmHg within 5 min on 26th day of the experiment for further research of neuroretinoprotective properties of pharmacological agents.

This model makes possible to evaluate the neuro- and retinotropic effects of new and commonly used pharmacological agents in ophtalmology sufficiently objectively.

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