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EFFECT OF PEPTIDES AND CITICOLINE ON EYE HEMODYNAMICS AND ENDOTHELIN LEVEL IN RETINITIS PIGMENTOSA

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Retinitis pigmentosa is the most common form of hereditary retinal dystrophy. To date, no approved therapy can stop the development of this disease or restore vision. The purpose of the study was to study hemodynamic parameters in the eye vessels and the level of endothelin-1 in the blood serum and tear fluid in patients with retinitis pigmentosa before and after using peptides and citicoline. 55 patients (110 eyes) diagnosed with retinitis pigmentosa aged 15–24 were examined. Doppler color flow mapping of the ophthalmic, central retinal, and posterior short ciliary arteries was performed. The level of endothelin-1 in blood serum and tear fluid was determined. The patients were treated with endonasal electrophoresis with Retinalamin and Omk1 instillation. All patients showed a statistically significant decrease in hemodynamic parameters in the central retinal artery and posterior short ciliary arteries and an increase in the level of endothelin-1. After treatment, hemodynamic parameters increase, and endothelin-1 levels decrease.

Key words: retinitis pigmentosa, Doppler color flow mapping, hemodynamics, endothelin-1, peptides, citicoline

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ВПЛИВ ПЕПТИДІВ І ЦИТИКОЛІНУ НА ГЕМОДИНАМІКУ ОКА ТА РІВЕНЬ ЕНДОТЕЛІНУ ПРИ ПІГМЕНТНОМУ РЕТИНІТІ

Пігментний ретиніт є найбільш поширеною формою спадкової дистрофії сітківки. На сьогоднішній день немає схваленої терапії, здатної зупинити розвиток цього захворювання або відновити зір. Метою дослідження було вивчити гемодинамічні параметри в судинах ока та рівень ендотеліну-1 у сироватці крові та слізній рідині у пацієнтів з пігментним ретинітом до та після застосування пептидів та цитиколіну. Було обстежено 55 пацієнтів (110 очей) з діагнозом пігментний ретиніт у віці 15–24 років. Проводили кольорове допплерівське картування очної артерії, центральної артерії сітківки та коротких задніх циліарних артерій. Визначався рівень ендотеліну-1 у сироватці крові та слізній рідині. Пацієнтам проводили курс терапії ендоназального електрофорезу з Ретиналаміном та інстиляції Омк1. У всіх пацієнтів виявлено статистично достовірне зниження гемодинамічних параметрів у центральній артерії сітківки, задніх коротких циліарних артерій та підвищення рівня ендотеліну-1. Після лікування підвищуються гемодинамічні параметри та знижується рівень ендотеліну-1.

Ключові слова: пігментний ретиніт, кольорове допплерівське картування, гемодинаміка, ендотелін-1, пептиди, цитиколін.

Retinitis pigmentosa (RP) belongs to the group of pigmentary retinopathy. This is the most common form of hereditary retinal dystrophy, characterized by progressive degeneration of photoreceptors, leading to nyctalopia and, ultimately, complete loss of vision. Typical signs of RP include retinal pigmentation in the form of bone spicules, narrowed retinal vessels and waxy pallor of the optic disc, which are called the classic triad. It is one of the leading causes of bilateral blindness in adults, with an incidence of 1 in 3000 people worldwide, but it can range from 1:9000 to 1:750 in different populations [3, 11]. RP is a big medical and social problem. RP's clinical, genetic and morphological heterogeneity makes it an extremely complex ocular lesion [4].

Various studies have found a decrease in blood flow in patients with RP. Based on a large number of studies of systemic and local reduction in blood flow parameters, it is assumed that insufficient blood circulation in the retina can change its chemical composition, leading to disruption of metabolism and the structure of cell membranes [8, 12, 15]. However, the role of vascular disorders in the development of the dystrophic process has practically not been proven. Questions remain to be answered regarding the causes of vascular dysfunction in RP and the diagnostic, prognostic and possibly therapeutic potential of studying ocular hemodynamics in patients with RP [7, 9]. In recent years, much attention has also been paid to the endothelin system in various eye pathologies and in cases of RP. Significant is the study of endothelin, a powerful vasoconstrictor peptide produced by vascular endothelial cells. It is one of the most significant regulators of the functional state of the vascular endothelium [13, 14].

To date, no approved therapy can stop the development of RP or restore vision. New therapeutic agents can be adapted depending on the stage of the disease and can be divided into three groups. In the early stages, when there are functioning photoreceptors, the first approach is to try to stop degeneration. The second approach aims to combat photoreceptor cell death using neurotrophic growth factors and limit oxidative damage. When there are few or no functioning photoreceptors in the late stages, strategies that can benefit include retinal transplantation, electronic retinal implants or optogenetic techniques to resensitize remnants of cone photoreceptor cells [10] genetically.

Thus, the development of treatments to prevent the disease is necessary. Although no treatment can effectively cure RP, the progression of the disease appears to be reduced in some cases through specific preventive approaches. In patients with dystrophic changes in the retina, the most effective drugs are those belonging to the group of biogenic peptides. The action of such drugs is based on the ability to positively affect the metabolic processes occurring in the tissues of the retina (tissue-specific effect on the retina). Clinical and experimental studies concerning the study of the effectiveness of the use of peptide drugs in ophthalmology have confirmed their pronounced neuro- and retinoprotective effect [1, 2]. Also, in the literature, there are works with the use of drugs based on citicoline, which has a neuroprotective effect in various eye pathologies. When administered in an ophthalmic solution, Citicoline can reach the human vitreous in high concentrations [5]. It enhances the regeneration of damaged cell surfaces, maintains cellular integrity and prevents apoptotic and necrotic cell death by reducing the content of phospholipases. Citicoline is effective in various neurodegenerative diseases of the retina and optic nerve [6]. However, the results can be considered only the first step towards its use in clinical practice to benefit patients.

The purpose of the study was to investigate hemodynamic parameters in the eye vessels and the level of endothelin-1 in the blood serum and tear fluid in young patients with retinitis pigmentosa before and after using peptides and citicoline.

Materials and methods. 55 patients (110 eyes) diagnosed with RP aged 15–24 (mean age 20.6±0.4) were examined. Patients with a severe cardiovascular or pulmonary disease, chronic or recurrent eye diseases (uveitis, scleritis, etc.), with an infectious lesion, eye injury or surgical intervention performed within the last 6 months, as well as with acute or progressive retinal disease were not included in the examination.

All patients underwent routine ophthalmological examinations: visometry, tonometry, biomicroscopy, perimetry, and ophthalmoscopy. Electroretinography (general, macular, rhythmic and cone) was performed to assess the retina's functional activity. The hemodynamic state of the eye was examined in all patients using Doppler ultrasound. Hemodynamic parameters were determined using Doppler color flow mapping (DCFM) of the eyeball and retrobulbar space vessels. The study was carried out on an ultrasound diagnostic system, "Nemio XG SSA-580A", from TOSHIBA with a linear sensor with a frequency of 8 MHz. To visualize blood flow in the ophthalmic artery (OA), central retinal artery (CRA) and short posterior ciliary arteries (SPCA), the energy mapping mode was used, and then the DCFM was switched on. The following hemodynamic velocity parameters were analyzed: maximum systolic velocity (Vmax), end-diastolic velocity (Vmin) and resistance index (RI). The control group for determining the reference values of hemodynamic parameters consisted of 50 healthy volunteers aged 15–24 years.

In all patients, the level of endothelin-1 (ET-1) in blood serum (BS) and tear fluid (TF) was determined. The study used a Stat Fax 2100 photometer (USA) using the enzyme immunoassay (ELISA) method. The control group consisted of 10 healthy people aged 15–24 years to determine the reference values of ET-1 levels. TF was collected using the irritation method using ordinary onion leaves. The duration of the procedure is approximately 5–10 minutes. TF was collected from the lower conjunctival sac using a Pasteur pipette.

Treatment of patients consisted of a course of therapy with endonasal electrophoresis (ENE) with Retinalamin and administration of Omk1 instillations. The course of ENE was 10 sessions for 2 weeks. ENE was carried out using a BTL 4000 electrotherapy device (Great Britain). The device has 2 electrodes, one indifferent and the other active (Fig. 1).
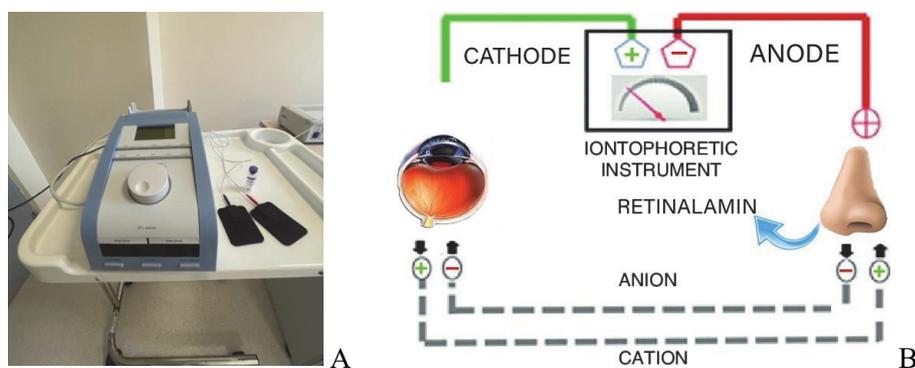

Fig. 1. Apparatus (A) and ENE scheme (B).

The indifferent electrode was fixed to the cervical vertebrae area, and the active electrode was fixed to the area of the upper lip on gauze swabs, previously soaked in Retinalamin solution and inserted into the nasal passages. The current strength was determined individually according to the patient's feelings. The duration of each therapy session ranged from 10 to 15 minutes. Citicoline instillations were carried out in a course of 4 months. Hemodynamic parameters and endothelin levels were studied before the use of drugs and 2 weeks after the ENE course.

Statistical processing of the data was carried out using the statistical package "SPSS 26" using the methods of variance analysis in dependent and independent groups with the calculation of the Mann-Whitney reliability index (pU), Wilcoxon (pW), as well as the Spearman correlation coefficient (ρ).

Results of the study and their discussion. Based on Doppler studies, a statistically significant decrease in blood flow parameters in the CRA and SPCA was observed in all patients with RP. There were no statistically significant changes in hemodynamic parameters in the OA compared to the control group before treatment (Table 1).

Table 1

Hemodynamic parameters before and after treatment

Arteries	Parameters	Standard	Before treatment	After treatment	pU	pW
OA	Vmax(cm/sec)	37.3±0.3	36.5±0.7	37.26±0.397	0.447	0.221
	Vmin(cm/sec)	9.47±0.12	9.49±0.33	9.81±0.162	0.976	0.230
	IR	0.75± 0.002	0.74±0.008	0.74±0.004	0.904	0.544
CRA	Vmax(cm/sec)	13.2±0.1	9.5±0.2	10.9±0.2	<0.001**	<0.001**
	Vmin(cm/sec)	3.82±0.04	3.15±0.06	3.37±0.04	<0.001**	<0.001**
	IR	0.71±0.002	0.657±0.007	0.685±0.004	<0.001**	<0.001**
SPCA	Vmax (cm/sec)	9.5±0.1	7.6±0.2	8.3±0.1	<0.001**	<0.001**
	Vmin (cm/sec)	3.67±0.04	3.25±0.06	3.36±0.04	0.001*	0.007*
	IR	0.61±0.002	0.553±0.01	0.587±0.002	<0.001**	<0.001**

* – p <0.05; ** – p <0.001 – statistically significant difference

After a course of ENE and citicoline installations, positive dynamics were observed according to the results of Doppler studies. Analysis of the DCFM data showed that of the 55 patients of the main group in the OA, the Vmax indicator was slightly increased in 26 patients, Vmin – in 27 and IR – in 24 patients. However, these changes in parameters in the OA turned out to be statistically unreliable. In the CRA, out of 55 patients, the Vmax parameter increased in 53, Vmin increased in 42, and IR – in 39 patients. In SPCA, all parameters also increased after treatment: Vmax in 32 patients, Vmin – in 19, and IR – in 31 patients. These changes were statistically significant.

Significant changes were observed in the CRA of the SPCA (Fig. 2 a, b and 3 a, b).
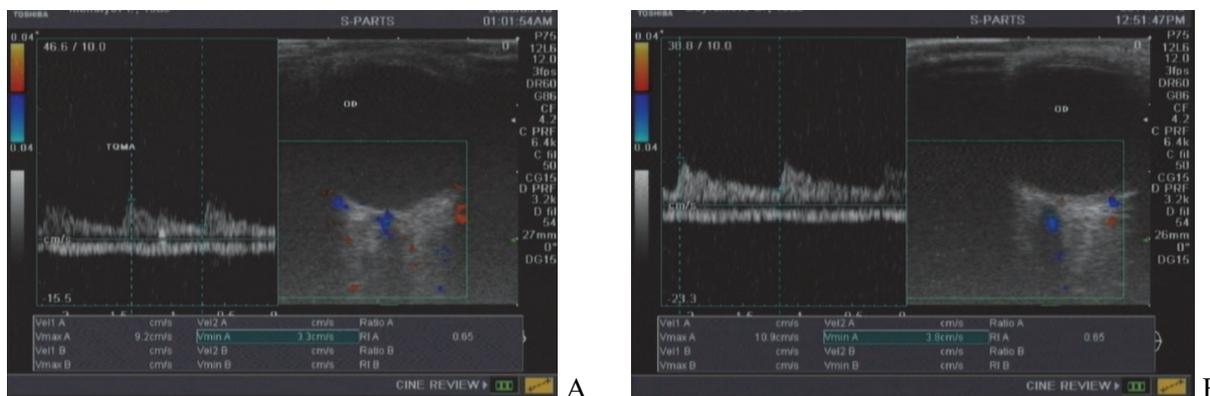

Fig. 2. Doppler ultrasonography of CRA. A – before treatment. B – after treatment.
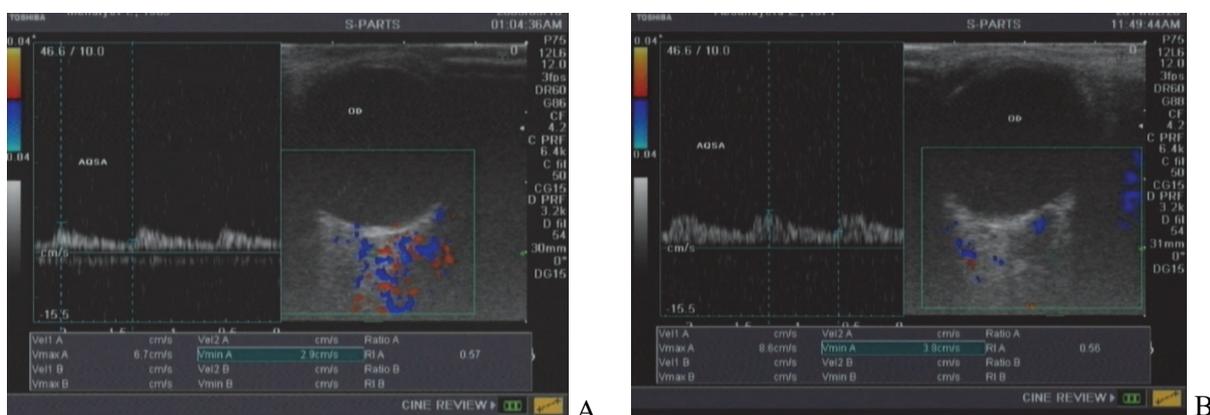

Fig. 3. Doppler ultrasonography of SPCA. A – before treatment. B – after treatment.

The ELISA results showed that before the use of the drugs, the level of ET-1 was statistically significantly increased in both the TF and the BS (Table 2).

Table 2

ET-1 level before and after treatment

ET-1 (fmol/mL)	Control	Before treatment	After treatment	pU	pW
TF	0.324±0.028	1.84±0.3	1.01±0.19	<0.001**	<0.001**
BS	0.324±0.028	0.43±0.084	0.212±0.036	0.005*	0.002*

* – p <0.05; ** – p <0.001 – statistically significant difference

Statistical processing of the results of the ET-1 study revealed positive dynamics after treatment. Thus, this parameter decreased in the TF in 27 patients by the mean of 1.8 times out of 29 examined and in the BS in the 21st patient by the mean of 2.0 times out of 26 examined.

Changes in ET-1 both before and after treatment became more pronounced in the TF. This result may indicate an ischemic state of the eyeball, more pronounced at the regional level.

Correlation analysis of Doppler indices and ET-1 level revealed the following results. There was a statistically significant inverse correlation between the level of ET-1 in the BS before treatment and the parameter Vmax in the CRA (ρ=-0.491. p=0.011) and Vmin in the CRA (ρ=-0.460. p=0.018). An inverse correlation was also revealed between ET-1 in the BS before treatment with the Vmax parameter in the SPCA (p=-0.442. p=0.024).

As a result of analyzing the available sources, we found several works that studied blood flow during RP. Thus, He et al. (2014) carried out OA DCFM, CRA and SPCA, and similar to our results, a more pronounced decrease in hemodynamic parameters was found in the CRA and SPCA [7]. There are some differences in the level of parameters, which is acceptable due to the different ages of the patients examined and population characteristics. Kayser et al. (2017) studied the mean blood flow velocity using DCFM and found a decrease in this parameter when central vision was also impaired due to the loss of functions of both rods and cones [8]. The work of Wang X.N. et al. (2019) is known, where DCFM of CRA and optical coherence tomography angiography (OCT-A) were performed in patients with RP to compare the respective advantages of these methods in the assessment of vascular changes [15]. According to the study's results, OCT-A detects changes in microvessels and thickness around the macula, correlating with visual acuity. Thus, OCT-A, like DCFM, is an informative method for diagnosing and monitoring patients with RP. Ling L. et al. (2019) studied the hemodynamics of the eye using OCT-A and revealed significant thinning of the retinal and choroid vessels in patients with RP [9]. Similar to ours, all these studies confirm retinal and choroid blood flow deterioration and suggest preventing or treating RP by maintaining normal ocular blood flow.

As for the study of ET-1, we did not find any studies examining the level of this indicator in the TF during RP. Considering that TF is an indicator of metabolic processes occurring in the eyeball, the study of TF more accurately reflects the state at the local level. However, the study by Strobbe et al. (2015) observed an increase in plasma ET-1 levels during RP, as in our work in the BS. The authors also identified a correlation between increased plasma ET-1 levels and decreased choroid thickness [14]. Based on an analysis of the literature, Sorrentino et al. (2015) concluded that impaired ocular blood flow and increased endothelin-1 levels in RP indicate ischemia typical of vascular dysregulation syndrome, which can be considered an important factor [13]. This fact further determines the need for a more detailed elucidation of the role of vascular disorders and ischemic conditions in the development of the dystrophic process as risk factors.

Khavinson et al. (2014) conducted electrophysiological and histological studies of patients with RP and experimental animals [2]. The authors concluded that peptide bioregulators help restore the physiological activity of the retina, improve the functional interaction of the pigment epithelium and the outer segments of photoreceptors, and regulate metabolic processes. In our study, we revealed the positive effect of peptides on the hemodynamics of the eye. Garcia-Lopez et al. (2023) provide data on positive results from using citicoline in pathologies such as glaucomatous optic neuropathy, diabetic retinopathy and age-related macular degeneration [6]. This justifies the use of citicoline in our study in patients with RP.

Conclusions

1. DCFM in patients with RP revealed hemodynamic disturbances in the eye vessels, significantly expressed in the CRA and SPCA with statistical significance.

2. As a result of studying the level of ET-1 in the BS and TF of patients with RP, a statistically significant increase in this parameter was observed, more significant in the TF.

3. Using peptides and citicoline is effective in patients with RP. As a result of treatment, hemodynamic parameters in the vessels of the eye increase and the level of ET-1 in the BS and TF decreases.

4. The data obtained indicate the presence of retinal ischemia, which, in turn, may play a role in the progression of the dystrophic process.

Thus, DCFM of the ocular vessels and study of the level of ET-1 in patients with RP may be an additional informative and non-invasive method for prognosis, assessment of severity and treatment control. The results of our study dictate the need for further research to elucidate the role of the vascular factor in RP fully.

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