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Molecular-Physiological Aspects of Regulatory Effect of Peptide Retinoprotectors

V. Khavinson[1,2] · S. Trofimova[1] · A. Trofimov[1] · I. Solomin[1]

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Abstract
Retinal diseases were always difficult problem for clinical ophthalmology. Modern methods of their treatment only decrease risk of complications, however in Russia was created better technology for this purpose: peptide bioregulators, which were made by sequential adding of amino acids one to another, binding with the promoter region of genes, and promoting retinoprotective effect by regulation of their expression, improving the state of the retina.

Keywords Short peptides · Peptide bioregulators · DNA · Retinal diseases · Binding sites · Promoter regions

Introduction

Retinal diseases (age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa) represent a difficult problem for clinical ophthalmology. According to data as of 2010 from the WHO, particularly retinal pathology, along with cancers, will be the leading cause of disability of the world population [1].

Modern methods of the treatment of retinal diseases—laser action, surgical and therapeutic treatment (such as Lucentis, Macugen, Visudyne) aim to just reduce the risk of complications in the eye. It must be noted that therapeutic medications (Lucentis, Visudyne, Macugen) are applied only during exudative forms of macular degeneration and diabetic retinopathy. Besides that, the application of these particular medicines can produce several contraindications (liver conditions, porphyfia, decompenasted hypertension, unstable angina, patients, taking sulfanilamides) and cannot be used in the whole population, because it may lead to severe complications and to an even bigger decrease of visual functions [2–6].

Numerous researches into the achievements of molecular biology are conducted at the present time for the purpose of stopping the progression of retinal pathology. Several scientists in their works provide a method for the restoration of functional activity of retinal neurons by replacement therapy with neuronal stem cells [7]. However all these works are experimental and have not been applied in clinical practice yet [8–11].

Thus, effective and tested methods for the treatment of retinal pathology in global ophthalmology do not exist at the present time. Unlike in Russia, where the pathogenetic treatment of retinal diseases with complex of peptide bioregulators, was developed back in 1970s in the S. M. Kirov Military Medical Academy.

This new direction of clinical medicine—bioregulating therapy, has received intensive development at the end of the twentieth century.

Bioregulating Therapy

A truly new approach to the creation of peptide drugs was suggested by V. Khavinson and V. Morozov. The authors developed and used an original method of extraction of low-molecular-weight peptides, which were given the umbrella term “cytomedines”, possessing high tissue-specificity, from organs and tissues. The concept of peptide bioregulation with the participation of endogenic peptide bioregulators in the maintainance of structural and functional homeostasis of cell populations, was formulated by these authors [12]. Peptides in accordance with this suggested concept are capable of stimulating the expression of cell differentiation markers by binding promoter regions of genes. It is necessary for the development, the interaction and the functioning of cells [13].

✉ I. Solomin
solomivan@gmail.com

[1] Saint Petersburg Institute of Bioregulation and Gerontlogy, Saint Petersburg, Russia

[2] Pavlov Institute of Physiology RAS, Saint Petersburg, Russia

The first peptide drug (retinalamin), possessing retinoprotective effect, was created 30 years ago in the S. M. Kirov Military Medical Academy. Retinalamin currently is widely used for the treatment of different retinal pathology (Minister of Healthcare of the Russian Federation order dated 24th December 2012 No. 1520n) and was included as standard retinal pathology treatment.

Also an important achievement is the development of the synthetic production of a new group of bioregulators—cytogens, from amino acids. Several short peptides, possessing a powerful retinoprotective effect,(Ala-Glu-Asp-Gly, Lys-Glu and other) were synthesized by using amino acid analysis of retinalamin.

Multi-year experimental researches into retinal peptides showed a high retinoprotective activity of these particular drugs. It is proved that peptide bioregulators have a pronounced therapeutic effect on models of toxic retinal degeneration, which appeared as a reduction in the size of dystrophic focus, the decline of retinal edema, and was also confirmed by histological studies [14].

Results of the researches, conducted on Campbell line rats, a characteristic of which is the development of genetically determined retinitis pigmentosa from the 20th day of life, also proved the high retinoprotective activity of peptides. Experimental research indicated that an enhancement of retinal functional activity by 2.8 times, according to the results of electroretinograms, was registered from the use of peptide bioregulators in animals with genetic retinal degeneration. Besides that, the retinoprotective activity of peptide promoted a 2-fold elongation of the maintenance period of morphological retinal structure in the main group of rats in comparison with the control group. Thus, peptide bioregulators are capable of suppressing the genetically programmed death of pigment epithelium cells and stimulate processes of regeneration of the retinal neuroreceptor system and restore its function [15–17].

The underlying mechanism of the action of peptide retinoprotectors can be characterized by the results of the following experimental researches.

It is known that the retina possesses up to 30% of undifferentiated cells (stems cells). Multi-year studies of mechanisms of action of peptide bioregulators in the Institute of Gene Biology (IGB) of the Russian Academy of Sciences (Russia, Moscow) in the laboratory of professor Lopashov G.V., showed that peptides possess strictly specific inductive activity. It was proved, during research into the inductive activity of retinal peptides on cells of pluropotent tissues of early gastrula ectoderm of *Xenopus laevis*, that these peptides possess neural inductive activity as opposed to other bioregulators, which possess, for example, mesodermal activity. Neural differentiation begins after the impact of retinal peptides on undifferentiated tissue. It leads to the appearance of neural cells, the retina and the pigment epithelium [18, 19]. Further research confirmed that short peptides possess inductive activity on undifferentiated stem cells [20]. Thus, it clearly revealed discovery one of the mechanisms of action of retinal peptides, explaining what it is possible to enhance in clinical practice, the visual functions in patients with eye diseases.

Molecular Mechanisms

Besides that, it is known that the molecular mechanism of the functional activity decline of retinal cells underlies pathological processes in the retina. The change of the expression of differentiation markers, participating in the ontogeny of the retina, is a key factor of the dyscrasia and the development of different dystrophic processes in it. Results from recent research indicate that short peptides stimulate the differentiation of neurons and cells of the retinal pigment epithelium (RPE). For instance, according to the results of immunocytochemical studies, it was registered that dipeptide (Lys-Glu) and tetrapeptide (Ala-Glu-Asp-Gly) are inductors of retinal cell differentiation. It is compliant with the results of other researchers into the stimulated effect of hormones and peptides on their functional activity. For instance, it is known that a change in the somatostatin expression level has an affect on the differentiation of bipolar cells [21]. Besides that, according to the literature data, it is known that several peptides ADNF-9 and NAP also promote an increase in the survival of ganglion cell culture, and also the stimulation of axon growth in the retinal explants [22].

It must be noted that dipeptide (Lys-Glu) to a greater degree affects the expression of the pigment epithelium cell marker TTR, which suggests the application of this particular peptide during the treatment of tapetoretinal abiotrophy, cone-rod retinal dystrophy and several other neurodegenerative diseases. While tetrapeptide (Ala-Glu-Asp-Gly) produces the most pronounced stimulation on the expression of the transcription factors Vsx1 (marker of the initial and the terminal differentiation of retinal bipolar cells), Pax6 (marker of the differentiation of pluripotent cells—predecessors of the retina) and Brn3 (marker of the differentiation of ganglion cells), which suggests the application of this particular peptide during the treatment of diabetic retinopathy and macular retinal degeneration [23, 24].

Besides that, it was established that tetrapeptide Ala-Glu-Asp-Gly penetrates into the cytoplasm, the nucleus and the nucleous of cells and regulates the methylation of the genes and the acivity of endonucleases [25–27]. In accordance with these results an assumption was made that tetrapeptide can bind with DNA.

Computer Modelling

A model of the interaction of tetrapeptide with promoter regions of genes, regulating the retinal cell differentiation, was built for the confirmation of this assumption.

A method of molecular dynamic, allowing the motion of individual molecules to be reproduced in specified time, was used to preselection the most energetically favorable conformation of tetrapeptide Ala-Glu-Asp-Gly. The main results were obtained in the force field MM+. Around 100 peptide preconformations were analyzed in every mode and then the optimal energy of rotamers chosen.

Computer modelling of the interaction of tetrapeptide Ala-Glu-Asp-Gly with DNA region, possessing assumed binding site ATTTC, was conducted. Calculations of complexes were conducted in the force field AMBER99. The energy of binding peptide with DNA (kcal/mol) was calculated as the energy differential of individual DNA molecules, peptide and DNA-peptide complex. It is established that tetrapeptide interacts with the major groobe of the DNA double helix. It is possible, that the interaction between tetrapeptide and 5'-ATTTC-3′ sequence and complementary to it 5′-GAAAT-3′ performs with the help of van der Waals forces, electrostatic interactions and hydrogen bonds among functional groups of both molecules [28, 29]. ATTTC sequence, which is complementary to tetrapeptide was found in the promoter region of genes Vsx1, Chx10, Pax6, Brn3, Math1, Prox1, TTR.

Study results indicate that tetrapeptide stimulates the expression of markers of the differentiation of retinal and pigment epithelium cells by binding with promoter regions of genes, which in turn leads to the restoration of the inner nuclear layer and the pigment epithelium.

So, the knowledge of these particular molecular mechanisms allows a targetted approach to the treatment of different retinal pathology depending on the main location of the lesion in the retina and develop an algorithm of the treatment of different retinal pathology (age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa) with the application a the complex of peptide bioregulators.

Results of the Application of Peptide Bioregulators

Thirty-years of the application of peptide bioregulators in clinical ophthalmology have showed, that the regular usage of peptide during the treatment of such retinal diseases, as retinitis pigmentosa, age-related macular degeneration, diabetic retinopathy, allows the progression of pathological process to be suppressed and provides an additional 10–15 years of sight to the patient. Besides that, the enhancement of visual functions in 80% of patients is shown due to the increase in visual acuity, in the improvement of field of vision limits and in the state of the eye fundus. Peptide drugs do not possess any side effect and do not provoke allergic reaction. [30–32]

Thus, the results of multi-year studies show the mechanisms of action of peptide retinoprotectors, and the pathogenetic proved application of peptide bioregulators in clinical practice makes them indispensable during the treatment of the retina.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict ofinterest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Statement of Human Rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statement on the Welfare of Animals All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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