Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Pineal-regulating tetrapeptide epitalon improves eye retina condition in retinitis pigmentosa

Vladimir Khavinson, Michael Razumovsky, Svetlana Trofimova, Roman Grigorian & Anna Razumovskaya

St. Petersburg Institute of Bioregulation and Gerontology of the North-Western Branch of the Russian Academy of Medical Sciences, RUSSIA.

Correspondence to: Prof. Vladimir Khavinson
St. Petersburg Institute of Bioregulation and Gerontology
3, Dynamo Prospect, 197110, St. Petersburg, RUSSIA
TEL./FAX: +7 812 2351832
E-MAIL: ibg@medport.ru

Submitted: *June 5, 2002*
Accepted: *June 14, 2002*

Key words: **peptide Epitalon; pineal gland; *Retinitis Pigmentosa*; Campbell rats; human patients**

*Neuroendocrinology Letters 2002; **23**:365–368 pii: NEL230402A11 Copyright © Neuroendocrinology Letters 2002*

Abstract We have studied the effect of tetrapeptide Epitalon (Ala-Glu-Asp-Gly) on the course of congenital pigmented degeneration of the retina. The application of Epitalon in Campbell rats is found to intensify the bioelectric and functional activity of the retina due to the preservation of its morphological structure. Epitalon therapy in patients with degenerative retinal lesions results in a positive clinical effect in 90% of the cases. The analysis of Epitalon effects suggests that the tetrapeptide participates in the mechanisms of transcription common for the epiphysis and retina.

Introduction

Treatment for *Retinitis Pigmentosa* is a topical and socially relevant problem. Degenerative processes of the retina are known to result from disturbed metabolism of specific proteins in the pigmented epithelium and other retinal layers [1,2].

In this connection, it appears crucial to develop and study pharmaceuticals, which can influence the structural and functional specialisation of retinal cells and, thus, provide a pathogenetic treatment for *Retinitis Pigmentosa*. Peptides of tissue-specific effect seem to be the most promising physiologically active substances. One of such compounds is tetrapeptide Epitalon (Ala-Glu-Asp-Gly) obtained by targeted chemical synthesis [3]. This peptide has been designed on the basis of the amino acid analysis of a complex pharmaceutical isolated from the retina of animals (Retinalamin). Retinalamin is included in the State Pharmacopoeia of the Russian Federation [4]. To synthesise this peptide we have used four amino acids detected in Retinalamin at the highest concentrations. It must be emphasised that the compositions of synthetic peptides designed according to the amino acid analyses of pharmaceuticals from the epiphysis (Epithalamin) and retina (Retinalamin) are identical. This identity can be explained by the common embryological origin of the epiphysis and eye retina, i.e., from the same progenitor cells of the anterior neural plate [5]. Moreover, pinealocytes and photoreceptor cells demonstrate a considerable similarity at the molecular level, in particular, in their transcription patterns [6,7,8]. These facts

suggest that Epitalon can restore retinal function, as well as the function of the pineal gland. Therefore, the purpose of our experimental and clinical investigations is to study the effect of Epitalon on the course of congenital pigmented retinal degeneration.

Material and methods

The experiments were carried out on Campbell rats provided by London University College of Surgery. Campbell rats are known to develop genetically determined retinal degeneration.

The experiments included 124 rats of different ages, which were examined from their birth to 72 days *postpartum*. The animals of the main group (62 rats) received parabulbar injections of Epitalon at the dose of 0.1 ml (1 μg) into each eye in the total volume of liquid of 0.2 ml per animal. The injections were made once a day every day for the whole experiment (72 days). The control animals (62 rats) were similarly administered with 0.2 ml of sterile 0.9% sodium chloride solution.

To assess the effect of Epitalon on the course of congenital *Retinitis Pigmentosa* in rats, electrophysiological and histological methods were applied. All experiments were carried out under anaesthesia of the animals with urethane administered parenterally at the dose of 1500 mg/kg. Electroretinogram (ERG) was registered with a 3-channel electrophysiological recorder in a screened dark chamber. After 10 minutes of adaptation to darkness, the animals were exposed to photoflashes at the minimal frequency of once per 5 sec. The intensity of the photoflashes was 500 Kd/m^2, and their duration was 50 msec. ERG was recorded in the main and control groups since Day 17 (by this time all the rats opened their eyes) until no more ERG responses could be obtained.

The animals of both groups were subjected to a morphological investigation. For this purpose, they were decapitated (4–5 rats in each series). The eyeballs were enucleated on Days 17, 29, 35, 41, 56, 59, 63 and 72 and fixed with 10% formalin in 96% alcohol. Paraffin-free sagittal sections of the eyeballs were stained with haematoxylin-eosin. The preparations were analysed at a ×700 magnification by computer-assisted image analysis. We defined the thickness of the inner plexiform layer, inner and outer nuclear layers and receptor layer.

At the clinic of the St. Petersburg Institute of Bioregulation and Gerontology 162 patients (324 eyes) aged 18–72 years with congenital *Retinitis Pigmentosa* were examined. Among them, there were patients with a newly revealed degenerative process and those with a 26 years long case history. The patients of the main group were injected with Epitalon parabulbarly at the dose of 5.0 μg into each eye daily once a day for 10 days (100.0 μg per course).

The control group included 46 patients (92 eyes) who were treated by conventional methods (vasodilators, angioprotectors, and antisclerotic agents) according to a standard protocol.

All patients gave their informed consent to participate in the trial. The data were processed statistically using "Statistica" software package.

Results

The retinal bioelectric activity of the Campbell rats was assessed by the average total activity of A-, B- and C-waves of ERG.

Table I demonstrates a significant decrease in the retinal bioelectric activity of the control rats by the 41st day *postpartum* and the absence of ERG responses by the 56th day. Under the effect of Epitalon, the main group animals preserved a relatively high amplitude of ERG waves until the 56th day *postpartum*. Thus by the 41st day, ERG amplitude in the experiment exceeded that in the control by 74.8% and, only on the 62nd day no ERG responses were registered in any of the main group animals.

Histological preparations of the retina demonstrated that at birth the morphological picture of the retina was similar in both groups. Changes in retinal layers and structures became detectable only after the 20th day *postpartum*. For example, in the control rats all retinal layers, i.e., nuclear, photoreceptor and outer plexiform layers (containing the synapses of rods and cones with horizontal and bipolar cells) became narrowed. Consequently, the inner nuclear layer comprising amacrine, bipolar and horizontal cells approximated the outer nuclear layer composed of cones and rods. These processes developed gradually. By Day 38, the control rats revealed an almost complete disappearance of the photoreceptor-containing layer or no response to haematoxylin-eosin staining. This may evidence the absence or a low quantity of photoreceptors or their chemical and physiologically weak activity, due to which the stain does not bind to the intracellular structures and cannot mark this part of the retina. The comparison of the morphological pictures of the experimental and control animals revealed a considerable difference between their retinal structures by Day 41 (see Figure). For instance, in the control rats we observed the complete destruction of all retinal layers, while the morphological picture in the main group rats showed the preservation of all retinal layers. Since Day 58 *postpartum,* the structure of photoreceptor layer in the main group rats was significantly disturbed. Although this layer of photoreceptors was still detectable at even later stages of the pathologic process, it was replaced with connective tissue, and the retina was completely destructed by Day 72. Thus, the comparison of the histological pictures demonstrates that Epitalon application helps to preserve retinal morphology in Campbell rats by 75.6% longer.

Taken together, the experimental results show that administration of Epitalon to rats with congenital *Retinitis Pigmentosa* increases their ERG amplitude by 74.8% by Day 41, and extends the period of preserved retinal morphology by 75.6% and of retinal functional activity by 43.9% as compared to the untreated controls.

Table I. Bioelectric activity of the retina in Campbell rats

Group	Average value of total A-, B- and C-wave amplitudes of ERG (μV) Days			
	35 (n = 12*)	41 (n = 14)	56 (n = 14)	59 (n = 16)
Control	96.6 21.3	49.2 10.6	0	0
Experiment	114.9 18.7	86.0 7.7**	69.4 15.6	37.5 10.5

n* – the number of examined eyes in each group;
**p<0.05 – in comparison with the control index.

The results of the experiments provided grounds for the clinical use of Epitalon in *Retinitis Pigmentosa* patients. All patients of the control and main groups had a decreased amplitude activity of the 1st and 2nd neurones and prolonged latency time of the 2nd neurone before the treatment. This corresponds to the results reported by Dryja *et al.* [9] and demonstrating that the primary defect in *Retinitis Pigmentosa* is localised at the level of photoreceptors.

Administration of Epitalon was accompanied by a significant rise in the amplitude activity of the 1st and 2nd neurones and by a reduced latency time of the 2nd neurone (see Table II). The higher functional activity of the retina resulted in an improvement in the visual functions of the main group patients. Visual acuity of the Epitalon-treated patients increased on average by 0.15–0.25. Moreover, in 7.6% of the cases (18 eyes) we registered a visual acuity increase by 0.4. The peripheral borders of the visual field extended in all the patients of the main group, and in 64.8% of them the total visual field borders (TVFB) increased by 90–120°. Absolute scotomas reduced in size, some of them became relative or disappeared after Epitalon administration.

The control group patients did not show any significant ERG changes, except for an increased latency time of the 2nd neurone. Improved visual functions (visual acuity, TVFB) were registered only in 31.5% of the cases, and the maximal attainable visual acuity increase amounted to 0.15 only in 4.3% of the cases. The rest of the control group patients showed either no improvement in their visual acuity or its decrease (in 4 eyes). An extended TVFB (by 40–50°) was observed in 86.9% of the cases. The results obtained in the control group evidence the insufficient effectiveness of the conventional therapy for peripheral pigmented retinal abiotrophy.

Discussion

The results reported in this article have a great practical value. Firstly, they provide a basis for the clinical use of Epitalon in congenital retinal degeneration. Secondly, they open prospects for a detailed study of interactions between the epiphysis and the retina.

The results of the experimental and clinical investigations of Epitalon effect on the course of congenital *Retinitis Pigmentosa* demonstrate a pronounced stimulating effect of the synthetic peptide upon the eye retina. Therefore, the use of Epitalon in patients with dystrophic lesion of the retina produces a positive clinical effect in more than 90% of the cases (visual acuity increase, extension of visual field borders, normalisation of electrophysiological indices). It is especially important that in none of the Epitalon-treated patients any exacerbation of their clinical picture was registered. Moreover, even persons with an unfavourable allergologic case history have not developed any side reactions, complications or other side effects after Epitalon therapy.

Earlier it has been found that the tetrapeptide restores the nocturnal peaks of melatonin in senescent *Rhesus* monkeys and enhances the secretory activity of radiation-damaged pinealocytes in rats [10, 11]. It must be emphasised that the spectrum of the known effects of Epitalon includes not only improved pineal and visual functions in rodents and primates but also prolonged lifespan in *Drosophila melanogaster* and mice [12, 13]. These data draw attention to the transcription factors, most of which are evolutionary conservative. For instance, the mammals have a DNA sequence TAATC(T) named "pineal regulatory element" or PIRE [7]. PIRE sequences are unique for promoters of genes

Table II. Amplitude activity (μV) and latency time (msec) of retinal neurones according to ERG results in Retinitis Pigmentosa patients

Index		Norm	Group: Control		Group: Experiment (Epitalon)	
			Before treatment	After treatment	Before treatment	After treatment
The 1st neurone	μV	30–60	19.9 1.1	18.8 1.1	22.4 1.7	27.3 1.6*
	msec	15–25	25.2 1.3	24.8 1.9	23.9 1.2	22.7 1.2
The 2nd neurone	μV	225–400	160.4 10.1	165.3 10.2	150.8 9.9	210.6 11.5*
	msec	37–50	75.5 4.8	89.1 5.1*	66.8 4.3	58.8 3.9

*p<0.05 – in comparison with the index before treatment.

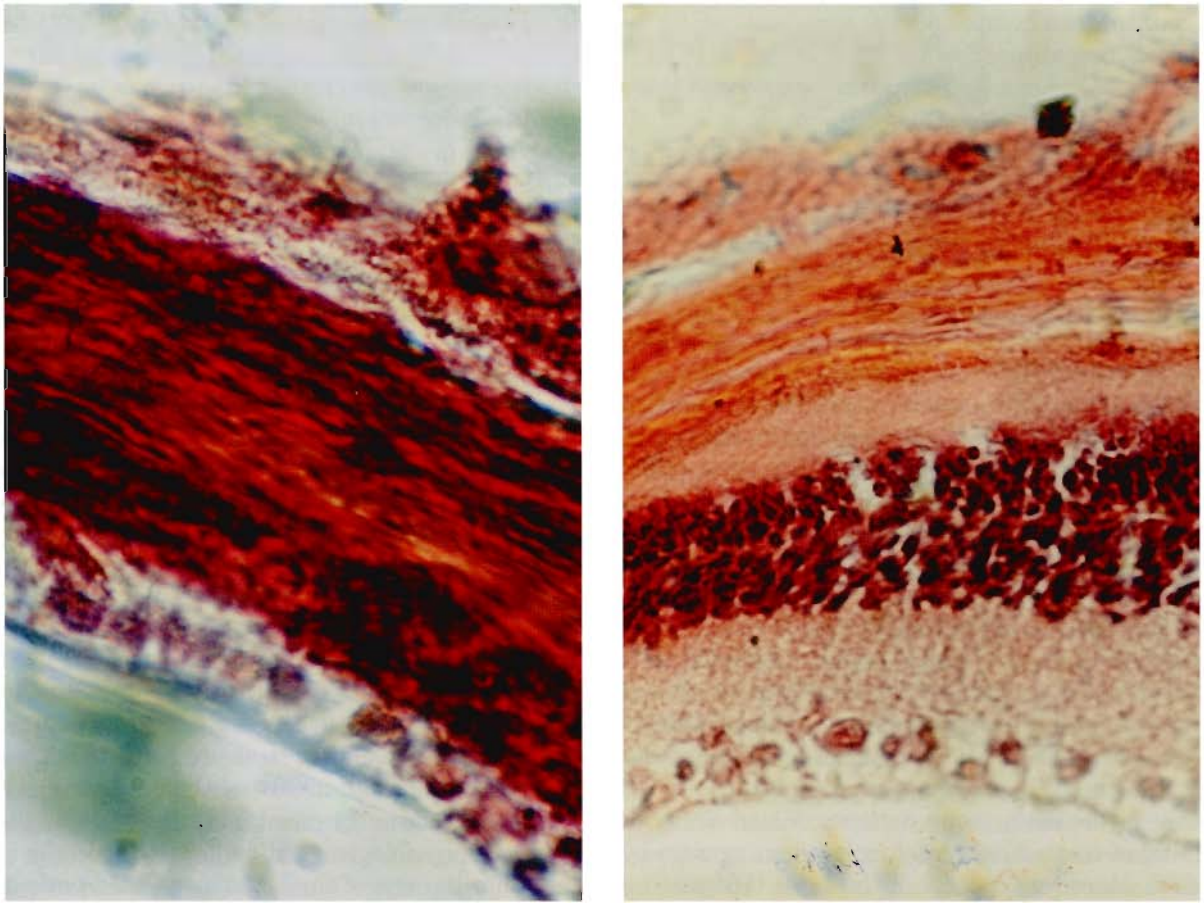

Figure. Morphologic picture of the retina in Campbell rats on the 41st day in the control (A) and in case of Epitalon administration (B). Haematoxylin-cosin staining, x700.

expressed in the retina (i.e., genes of photoreceptors) and in the epiphysis, in particular the gene of arylamine-N-acetyltransferase, a rate-limiting enzyme of melatonin biosynthesis. PIRE-binding transfactors are specific for the epiphysis and the retina. Mutations in the genes of some of these transfactors cause certain congenital forms of retinal degeneration [7].

Presumably, at least some of the known effects of Epitalon can be caused by its ability to bind either directly to PIRE or to some other epiphysis- and retina-specific DNA sites or to transfactors participating in the regulation of expression of the respective genes. In this way, Epitalon can exert a positive effect upon melatonin production in the pineal gland of the mammals and in *Drosophila melanogaster* and upon retinal functions.

REFERENCES

1 Cocburn D. Retinitis Pigmentosa: A review of the tapeto-retinal dystrophies. Amstr J Ophtomet 1985; **68**:138–143.
2 Friedman NJ, Pineda IIR, Kaiser PK. The Massachusetts eye and ear infirmary illustrated manual of ophthalmology. Philadelphia, USA; 1998.
3 Khavinson VKh. Tetrapeptide stimulating the function of the eye retina, a pharmacological substance on its basis and the method of its application. Patent of the Russian Federation No. 2161982; 20.01.2001.
4 Khavinson VKh. The method of obtaining peptides with tissue-specific activity and pharmaceutical compounds on their basis. Patent of the Russian Federation No. 2161501; 10.01.2001.
5 Melendez-Ferro M, Villar-Cheda B, Mannoel Abalo X, Perez-Costas E, Rodriquez-Munoz R, Degrip WJ, Yanez J, Rodicio MC, Anadon R. Early development of the retina and pineal complex in the sea lamprey: comparative immunocytochemical study. J Comp Neuro 2002; **442**:250–265.
6 Sohocki MM, Malone KA, Sullivan LS, Daiger SP. Localization of retina/pineal-expressed sequences: identification of novel candidate genes for inherited retinal disorders. Genomics 1999; **58**: 29–33.
7 Li XX, Chen S, Wang Q, Zack DJ, Snyder SH, Borjigin J. A pineal regulatory element (PIRE) mediates transactivation by the pineal/retina-specific transcription factor CRX. Proc Natl Acad Sci USA 1998; **95**(4):1876–1881.
8 Marmor MF, Wolfensberger TJ. The retinal pigment epithelium: function and disease. New York: Oxford University Press; 1998.
9 Dryja TP. VcGee TL, Reichel E. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature 1990; **343**:364–366.
10 Khavinson V, Goncharova N, Lapin B. Synthetic tetrapeptide Epitalon restores disturbed neuroendocrine regulation in senescent monkeys. Neuroendocrinology Lett 2001; **22**:251–254.
11 Khavinson VKh, Yakovleva ND, Popuchiev VV, Kvetnoi IM, Manokhina RP. Reparative effect of Epitalon on pineal gland ultrastructure in gamma-irradiated rats. Bull Exp Biol Med 2001; **131**(1):81–85.
12 Khavinson VKh, Izmaylov DM, Obukhova LK, Malinin VV. Effect of Epitalon on the lifespan increase in Drosophila melanogaster. Mech Ageing Dev 2000; **120**:141–149.
13 Anisimov VN, Khavinson VKh, Mikhalski AI, Yashin AI. Effect of synthetic thymic and pineal peptides on biomarkers of ageing, survival and spontaneous tumour incidence in female CBA mice. Mech Ageing Dev 2001; **122**:41–68.