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ANALYSIS OF THE RESULTS OF RETINAL PEPTIDE APPLICATION IN DIFFERENT AGES OF CAMPBELL RATS WITH RETINITIS PIGMENTOSA

U.F. Hashimova [1], A.N. Mammadzada [2]

[1] ***Academician Abdulla Garayev Institute of Physiology, Ministry of Science and Education of the Republic of Azerbaijan***, *78 Sharifzadeh Street, AZ1100, Baku, Azerbaijan*
[2] ***Academician Zarifa Aliyeva National Centre of Ophthalmology***, *32/15 Javadkhan Street, AZ1114, Baku, Azerbaijan*

E-mails: ulduz.hashimova@physiology.science.az; mamedzade04@mail.ru
ORCID ID: U.F. Hashimova 0000-0002-7740-0972

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Abstract:

In this paper, various age groups of Campbell rats with Retinitis Pigmentosa (RP) were studied for electrophysiological parameters and, based on histological examinations, morphological characteristics of the retina before and after Retinalamin treatment. 50 Campbell rats, as an experimental model of inherited RP, were used in the study. Rats were divided into two age groups, with 25 rats in each. Group I consisted of newborns up to 3 weeks, and Group II consisted of adults aged 3.5 ± 0.3 months. Retinalamin injections were given parabulbarly to all animals for 10 days. The total bioelectrical activity of the a, b, and c waves and the average value of the b-wave amplitude were studied using the electroretinography (ERG) method.

Also, the thickness of the outer nuclear layer of retinal pigment epithelium cells was studied in a histological examination. Studies were conducted before the start of Retinalamin injections and in dynamics after 10 and 30 days.

According to the ERG results, a decrease in the total bioelectrical activity of the rat retina and the average b-wave amplitude was already observed in 19-day-old rats. A statistically significant increase in these indicators was observed in both groups after 10 and 30 days of Retinalamin injections. Histological analysis of retinal preparations also revealed positive changes after the peptide administrations. An improvement in morphological structures and thickening of the outer nuclear layer of retinal pigment epithelial cells were noted in both groups, and more significant positive dynamics were in Group I of newborn rats.

Conclusion. Retinalamin treatment in newborn and adult Campbell rats with retinitis pigmentosa stabilized the processes developing in the retina, and the maximum positive effect was manifested in neonatal rats.

Keywords: Campbell rats, retinitis pigmentosa, ERG, Retinalamin

INTRODUCTION

Despite modern advances in science and medicine, hereditary diseases remain a serious pathology and are considered a medical and social problem since the treatment of such patients is difficult. One such pathology in ophthalmology is Retinitis Pigmentosa (RP), which is a genetically determined progressive disease of the vision organ with primary damage

to the photoreceptor layer and pigment epithelium. The prognosis of RP is considered unfavorable due to the constant development of the degenerative process, leading to complete blindness [3, 9, 13]. RP has no age limits, affects mainly young people, and leads to disability among the working population. The diagnosis of RP relies on data from electroretinography (ERG) identifying progressive loss of photoreceptor function. The use of ERG is aimed at early detection of ophthalmopathology and monitoring the effectiveness of the methods of treatment used. An in-depth ERG examination of the pathogenic processes and functional indicators of the progression of RP is becoming increasingly important.

The incidence of various retinal pathologies is constantly decreasing due to the development of methods of prevention, early diagnosis, and specific treatment. Still, in the case of blindness caused by RP, the problem remains acute. An analysis of the literature showed many multidirectional and diverse treatment methods for RP, but pathogenetic treatment does not exist today [3, 7]. Specific treatments are in development and clinical trials. Genetic experimental animal models are necessary for studying the development and functioning of the eye and various visual pathologies [8]. Experimental medicine shows promising results from gene therapy, stem cells, and other therapeutic methods for RP [1, 10, 11]. In clinical practice today, only maintenance therapy aimed at slowing the progression of the dystrophic process is used. One of the drugs that has a retinoprotective effect is Retinalamin. This drug belongs to the group of biogenic peptides; its action is based on its ability to positively affect the metabolic processes in the retinal tissue [5, 6, 12].

Considering the above, we decided to evaluate the effect of Retinalamin usage in RP in an experiment.

The aim was to study electrophysiological parameters, as well as, based on histological studies, morphological parameters of the retina in different age populations of Campbell rats before and after the use of peptides.

MATERIALS AND METHODS

The experimental object of our study was 50 Campbell rats (Fig. 1). These are the Royal College of Surgeons (RCS) linear rats - a classic model object for studying hereditary retinal pigmentary dystrophy. Campbell rats are pale-hooded, pink-eyed, homozygous individuals with hereditary retinal dystrophy and a disorder of the specific phagocytic function of the retinal pigment epithelium.

The rats were placed in a vivarium under comfortable conditions (ambient temperature of about 21°C).

To feed the animals, complete food for rodents was used with additional complementary foods of fruits and vegetables. The animals consumed water from drinking bowls. Water and food were freely available to the rats. Routine cleaning of the cages was carried out daily. General cleaning and disinfection of cages were performed weekly.

Two age groups of 25 rats each were identified for our investigation. Group I consisted of newborns up to 3 weeks, and Group II consisted of adults aged 3.5±0.3 months. The body weight of rats was determined using the Sartorius ED423S-RCE laboratory scale (Germany). All animals received parabulbar injections of Retinalamin for 10 days (Fig. 1). Studies were conducted both before the start of injections and overtime after 10 and 30 days to observe the effect of exposure.

Figure 1. Parabulbar injections.

ERG was used in the experiment to study electrophysiological parameters and evaluate the retina's functional state. Currently, ERG

remains the leading method for studying the functional signs of the development of RP. This research method makes it possible to identify not only pronounced dystrophic changes in the retina but also to diagnose biochemical and functional disorders that precede the clinical manifestations of RP [2, 14]. Electrophysiological parameters were recorded on special modules of the Neuro-ERG electrophysiological complex (Neurosoft LLC, Russia) (Mydriacyl, Alcon). After this, the animals were adapted to darkness for 30 minutes. ERG recording was performed using a corneal contact electrode (loop active electrode). The reference needle electrode was placed subcutaneously on the back of the animal's neck. The total bioelectrical activity of waves a, b, and c and the average amplitude of the b-wave were determined. Separate quantitative determination of other ERG components (a-wave and off-response) was weakly expressed in these experimental animals, so it was not taken into account.

To study morphological parameters, a histological examination of the retina was carried out, and the thickness of the outer nuclear layer of retinal pigment epithelium cells was studied. The rats were sacrificed, the eyeballs were removed, the anterior chamber was opened, and the eyes were fixed by immersion in a solution of 4% paraformaldehyde for 24 hours at a temperature of 4°C. Then the eyes were soaked in a 30% sucrose solution for 24 hours at a temperature of 4°C and frozen at a temperature of -24°C, after which sections 20 μm thick were prepared using a Leica cryotome model CM 1510S (Germany). The sections were then mounted on glass slides and immunohistochemically stained. Nuclear staining using the fluorescent dye bisbenzimide, which stains the nuclei of living cells, was used as background staining. Measurement of the thickness of the outer nuclear layer (ONL) was carried out on fluorescent micrographs of stained sections taken in the light of bisbenzimide fluorescence using an Olympus IX81 microscope. 10 rats (5 from each age group) made up the control groups; they were sacrificed without prior drug administration to study the retina's histological structure for further comparative analysis.

The remaining 40 rats (20 from each group) were sacrificed 30 days after the application of the peptides to identify histological changes.

To assess the reliability of the results obtained, the Newman-Keuls criterion was used. The results obtained were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

The ERG results revealed a decrease in the total bioelectrical activity of the retina of rats with RP already in 19-day-old rats. Thus, before the use of peptides in group I, this indicator was on average 88.6 ± 10.1 μV, and in group II in adult rats, accordingly, this indicator was even lower — on average 10.5 ± 3.8 μV. After injections of Retinalamin, a statistically significant increase in the total bioelectrical activity of the retina was observed in both groups (Fig. 2). In group I, an increase of 25.5% was observed after 10 days and another 13.6% after 30 days.

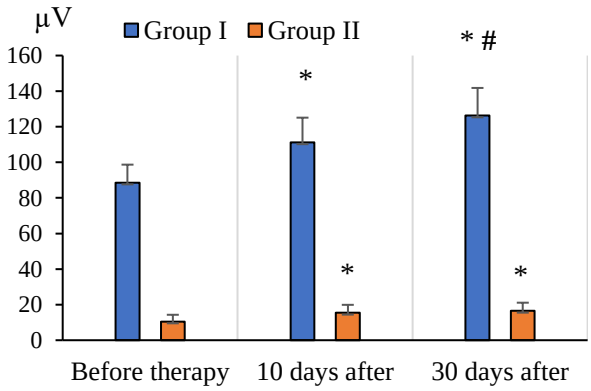

Figure 2. The total bioelectric activity of the a, b, and c ERG waves of rats before and after using Retinalamin (M ± m, μV)
* - $p < 0.05$ – a statistically significant difference compared to the parameter before the peptide's application. # - 0.05 – statistically significant difference compared to the previous data

In – group II, after 10 days there was an increase of 46.7%, and after 30 days there was another 7.1%. Analysis of the average b-wave amplitude also showed positive dynamics (Fig. 3). After applying peptides to group I, after 10

days, this indicator increased by 10.6%, and after 30 days, an increase of 19.4% was also observed. In group II, after 10 days, there was an increase in the average b-wave amplitude of 9.7%, and after 30 days, by 6.3%. These changes were statistically significant ($p<0.05$).

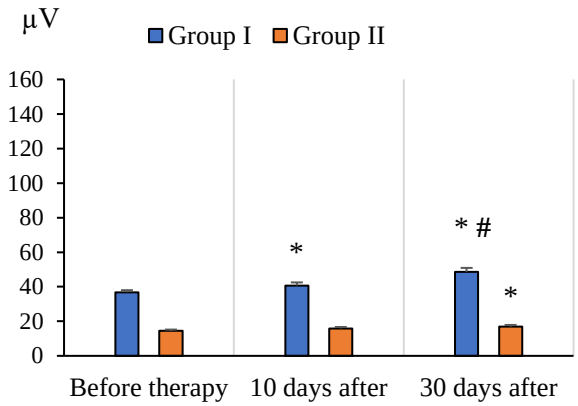

Figure 3. The average amplitude of ERG b-wave in rats before and after using Retinalamin (M ± m, μV)

* - $p < 0.05$ – a statistically significant difference compared to the parameter before the peptide's application. # - $p < 0.05$ – statistically significant difference compared to the previous data

Morphological analysis of retinal preparations also revealed positive changes after using peptides. Examination of sections in both groups of rats before injections revealed degenerative changes, more pronounced in group II. Destruction of the layers was observed, as well as thinning of the outer nuclear layer of retinal pigment epithelial cells (Fig. 4, 6, and Table 1).

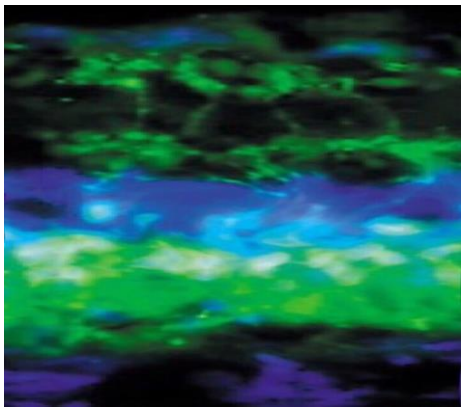

Figure 4. Retinal section before therapy in Group I. (zoom 1:400)

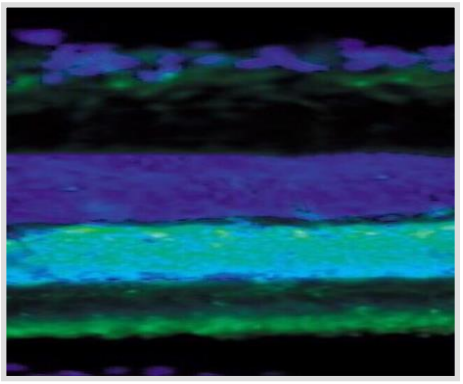

Figure 5. Retinal section after therapy in Group I. (zoom 1:400)

The severity of the lesion in terms of the area of degeneration foci in group I averaged 36.5±5.2%, and in group II, it was 68.2±6.3%. After the injections, an improvement in morphological structures and thickening of the outer nuclear layer of retinal pigment epithelium cells was observed on sections in both groups (Fig. 5, 7, Table 1). In group I, the average thickness of the outer nuclear layer increased statistically significantly by 17.3% ($p<0.05$), and in group II, by 10.8% ($p<0.05$).

Table 1. The average thickness of the outer nuclear layer of retinal pigment epithelium cells before and after the use of Retinalamin (M ± m, μm).

Age groups of rats	Before therapy (n=5)	30 days after (n=20)
Group I	29.4±1.1	34.5±1.2*
Group II	25.1±1.0	27.8±1.1*

* - $p < 0.05$ – a statistically significant difference compared to the parameter before application of peptides

Analyzing the literature sources, we found several works that conducted studies similar to ours. According to the study, which also used ERG on an experimental model of RP in Campbell rats, the b-wave amplitude increased with increasing stimulus brightness on the 20th day after birth. On day 30, the b-wave amplitude

in Campbell rats was much smaller than in Wistar rats and did not change with increasing stimulus brightness [4]. Another electrophysiological study of patients with RP and experimental animals determined that peptide bioregulators helped 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 [6].

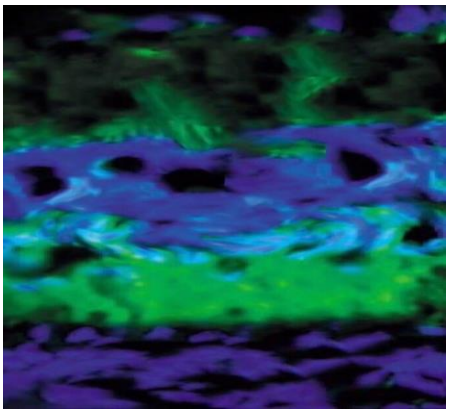

Figure 6. Retinal section before therapy in Group II. (zoom 1:400)

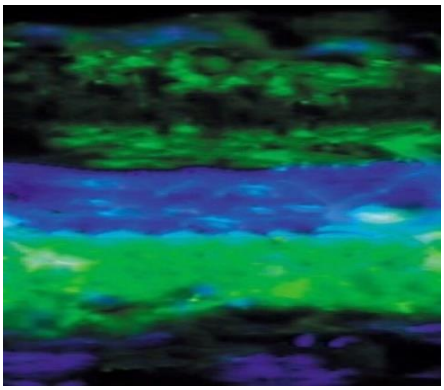

Figure 7. Retinal section after therapy in Group II. (zoom 1:400)

The use of Retinalamin led to a significant increase in the ERG b-wave compared to the control. Thus, in cases of moderate severity of retinal damage, the therapeutic effect of Retinalamin was detected on the 15th day of observation, reaching its maximum by 35 days. The amplitude of the ERG b-wave in experimental animals during this period reached 80% of the background level and was almost two times higher than that in the control group [5].

All these data correspond to the results of our study and prove the positive effect of Retinalamin on the retina. Also, the positive effect of the peptides in our experiment was confirmed by a histological study of the retina.

CONCLUSION

In a group of adult and neonatal Campbell rats with RP, the application of Retinalamin stabilized the processes developing in the retina. This was demonstrated by improvements in the histological features of the retina as well as a statistically significant increase in ERG indicators. In this case, neonatal rats show the most pronounced effect.

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АНАЛИЗ РЕЗУЛЬТАТОВ ПРИМЕНЕНИЯ ПЕПТИДОВ СЕТЧАТКИ В РАЗНЫХ ВОЗРАСТНЫХ ГРУППАХ КРЫС ЛИНИИ CAMPBELL С ПИГМЕНТНЫМ РЕТИНИТОМ

[1] Улдуз Фаизи кызы Гашимова, [2]Афет Насиб кызы Мамедзаде

[1] Институт физиологии им. академика Абдуллы Гараева, Министерство науки и образования Азербайджанской Республики, Баку, Азербайджан
[2] Национальный Офтальмологический Центр им. академика Зарифы Алиевой

Цель – изучить электрофизиологические показатели, а также морфологические показатели сетчатки в разных возрастных группах крыс линии Campbell с пигментным ретинитом (ПР) до и после применения ретиналамина.

В эксперименте были использованы 50 крыс линии Campbell, являющиеся экспериментальной моделью наследственного ПР. Были выделены 2 возрастные группы крыс по 25 в каждой. I группу составили новорождённые особи до 3-ех недель, II группу – взрослые особи в возрасте 3,5±0,3 месяца. Всем животным проводили парабульбарные инъекции ретиналамина в течение 10-ти дней. Методом электроретинографии (ЭРГ) изучали суммарную биоэлектрическую активность волн a, b, c и среднее значение амплитуды b-волны. Также проводили гистологическое исследование и изучали толщину наружного ядерного слоя клеток пигментного эпителия сетчатки. Исследования проводились до начала инъекций ретиналамина и в динамике через 10 и 30 дней.

По результатам ЭРГ наблюдалось снижение суммарной биоэлектрической активности сетчатки крыс и среднего значения амплитуды b-волны уже в возрасте 19 суток. После инъекций ретиналамина наблюдалось статистически достоверное повышение этих показателей в обеих группах через 10 и 30 дней. Морфологический анализ препаратов сетчатки выявил также положительные изменения после применения пептидов. Наблюдалось улучшение морфологических структур и утолщение наружного ядерного слоя клеток пигментного эпителия сетчатки в обеих группах.

Заключение. Применение ретиналамина у новорожденных и взрослых особей крыс линии Campbell с пигментным ретинитом оказывает стабилизирующее влияние на процессы, развивающиеся в сетчатке. При этом максимальный положительный эффект проявляется у новорожденных крыс.

Ключевые слова: крысы линии Campbell, пигментный ретинит, ЭРГ, ретиналамин

PİQMENTLİ RETİNİTİ OLAN CAMPBELL CİNSLİ SİÇOVULLARIN MÜXTƏLİF YAŞ QRUPLARINDA TORLU QİŞA PEPTİDLƏRİNİN TƏTBİQİNİN NƏTİCƏLƏRİ

Ulduz Fayızi qızı Həşimova [1], Afət Nəsib qızı Məmmədzadə [2]

[1] *Akademik Abdulla Qarayev adına Fiziologiya İnstitutu, Azərbaycan Respublikası Elm və Təhsil Nazirliyi, Bakı, Azərbaycan*
[2] *Akademik Zərifə Əliyeva adına Milli Oftalmologiya Mərkəzi*

Məqsəd – Campbell cinsli siçovulların müxtəlif yaş populyasiyalarında retinalaminin tətbiqindən əvvəl və sonra elektrofizioloji və histoloji tədqiqatlar əsasında tor qişanın morfoloji göstəricilərinin öyrənilməsi.

Eksperimentdə irsi piqmentli retinitin (PR) modeli olan Campbell cinsli 50 sıçovul istifadə olunmuşdur. Sıçovullar, hərəsində 25 olmaqla, 2 yaş qrupuna bölünmüşdür. I qrupu 3 həftəlik yenidoğulmuş, II qrupu 3,5±0,3 aylıq yetkin sıçovullar təşkil etmişdir. 10 gün ərzində bütün sıçovullara retinalaminin parabulbar inyeksiyaları aparılmışdır. Elektroretinoqrafiyanın (ERQ) köməyi ilə a, b, c dalğalarının ümumi bioelektrik aktivliyi və b-dalğasının amplitudasının orta dəyəri öyrənilmişdir. Histoloji tədqiqatlar əsasında tor qişanın piqmentli epitel hüceyrələrinin xarici nüvə qatının orta qalınlığı öyrənilmişdir. Müayinələr Retinalamin inyeksiyalarından əvvəl, və dinamikada10 gün və 30 gün sonra aparılmışdır.

ERQ nəticələrinə görə artıq 19 sutkalıq sıçovullarda a, b, c dalğalarının ümumi bioelektrik aktivliyinin və b-dalğasının amplitudasının orta dəyərinin azalması müşahidə edilmişdir. Retinalamin inyeksiyalarından 10 və 30 gün sonra hər iki qrupda bu göstəricilərin statistik etibarlı yüksəlməsi qeydə alınmışdır. Peptidlərin tətbiqindən sonra tor qişanın preparatlarının histoloji müayinəsi əsasında da müsbət dəyişikliklər təsdiqlənmışdır. Morfoloji strukturların yaxşılaşması və tor qişanın piqmentli epitel hüceyrələrinin xarici nüvə qatının orta qalınlığının artması müşahidə edilmişdir.

Yekun. Campbell cinsli siçovulların yenidoğulmuşlar və yetkinlər populyasiyasında retinalminin istifadəsi tor qişada baş verən proseslərə stabilləşdirici təsir göstərir. Müsbət dəyişikliklər daha əhəmiyyətli dərəcədə yenidoğulmuş sıçovullarda özünü göstərir.

Açar sözlər: Campbell cinsli siçovullar, piqmentli retinit, ERQ, retinalamin

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