



Effects of subtenon-injected autologous platelet-rich plasma on visual functions in eyes with retinitis pigmentosa: preliminary clinical results

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Received: 29 June 2017 / Revised: 26 February 2018 / Accepted: 2 March 2018
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Abstract

Purpose One of the main reasons for apoptosis and dormant cell phases in degenerative retinal diseases such as retinitis pigmentosa (RP) is growth factor withdrawal in the cellular microenvironment. Growth factors and neurotrophins can significantly slow down retinal degeneration and cell death in animal models. One possible source of autologous growth factors is platelet-rich plasma. The purpose of this study was to determine if subtenon injections of autologous platelet-rich plasma (aPRP) can have beneficial effects on visual function in RP patients by reactivating dormant photoreceptors.

Material and methods This prospective open-label clinical trial, conducted between September 2016 and February 2017, involved 71 eyes belonging to 48 RP patients with various degrees of narrowed visual field. Forty-nine eyes belonging to 37 patients were injected with aPRP. A comparison group was made up of 11 patients who had symmetrical bilateral narrowed visual field (VF) of both eyes. Among these 11 patients, one eye was injected with aPRP, while the other eye was injected with autologous platelet-poor plasma (aPPP) to serve as a control. The total duration of the study was 9 weeks: the aPRP or aPPP subtenon injections were applied three times, with 3-week intervals between injections, and the patients were followed for three more weeks after the third injection. Visual acuity (VA) tests were conducted on all patients, and VF, microperimetry (MP), and multifocal electroretinography (mfERG) tests were conducted on suitable patients to evaluate the visual function changes before and after the aPRP or aPPP injections.

Results The best-corrected visual acuity values in the ETDRS chart improved by 11.6 letters (from 70 to 81.6 letters) in 19 of 48 eyes following aPRP application; this result, however, was not statistically significant ($p = 0.056$). Following aPRP injections in 48 eyes, the mean deviation of the VF values improved from -25.3 to -23.1 dB ($p = 0.0001$). Results regarding the mfERG P1 amplitudes improved in ring 1 from 24.4 to 38.5 nv/deg² ($p = 0.0001$), in ring 2 from 6.7 to 9.3 nv/deg² ($p = 0.0301$), and in ring 3 from 3.5 to 4.5 nv/deg² ($p = 0.0329$). The mfERG P1 implicit times improved in ring 1 from 40.0 to 34.4 ms ($p = 0.01$), in ring 2 from 42.5 to 33.2 ms ($p = 0.01$), and in ring 3 from 42.1 to 37.9 ms ($p = 0.04$). The mfERG N1 amplitudes improved in ring 1 from 0.18 to 0.25 nv/deg² ($p = 0.011$) and in ring 2 from 0.05 to 0.08 nv/deg² ($p = 0.014$). The mfERG N1 implicit time also improved in ring 1 from 18.9 to 16.2 ms ($p = 0.040$) and in ring 2 from 20.9 to 15.5 ms ($p = 0.002$). No improvement was seen in the 11 control eyes into which aPPP was injected. In the 23 RP patients with macular involvement, the MP average threshold values improved with aPRP injections from 15.0 to 16.4 dB ($p = 0.0001$). No ocular or systemic adverse events related to the injections or aPRP were observed during the follow-up period.

Conclusion Preliminary clinical results are encouraging in terms of statistically significant improvements in VF, mfERG values, and MP. The subtenon injection of aPRP seems to be a therapeutic option for treatment and might lead to positive results in the vision of RP patients. Long-term results regarding adverse events are unknown. There have not been any serious adverse events and any ophthalmic or systemic side effects for 1 year follow-up. Further studies with long-term follow-up are needed to determine the duration of efficacy and the frequency of application.

Keywords Retinitis pigmentosa · Growth factors · Platelet-rich plasma · Visual functions

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Introduction

More than 240 genetic mutations are involved in inherited retinal dystrophies, which constitute an overlapping group of genetic and clinical heterogeneous disorders [1, 2]. Retinitis pigmentosa (RP) is a heterogeneous genetic disorder (autosomal dominant, autosomal recessive, X-linked, or sporadic cases from spontaneous mutations) characterized by the progressive devolution of the retina and affecting 1/3000–8000 people worldwide [1–4]. Symptoms include generally diminishing visual fields starting in the mid-periphery and advancing toward the fovea, ultimately leading to visual impairment and blindness with waxy-colored optic atrophy [4]. RP is also described as rod-cone dystrophy because of the primary degeneration of rods along with secondary degeneration of cones, with photoreceptor rods appearing to be more affected than cones [3]. Diseased photoreceptors face apoptosis, which results in reducing the thickness of the outer nuclear layer and the retinal pigment epithelium layer with abnormal pigmentary deposits [5]. Although apoptosis and photoreceptor loss are common outcomes of all genetic types, their clinical features and progression are not homogeneous [6, 7]. It is currently known that while some photoreceptor cells do die, others appear to be in “suspended animation” [8].

In the photoreceptor microenvironment, when growth factor (GF) levels or their receptor activities decrease over an extended period, apoptosis and cell death occur. The length of this period differs with each genetic type. The time during which there is a decrease in the effects of growth factors until cell death, the photoreceptors can be described as being in sleep mode, on standby, or in a dormant phase. In this phase, cone photoreceptors are alive, but they cannot function [9–13].

GFs and neurotrophins, such as basic fibroblast growth factor (bFGF), neural growth factor (NGF), ciliary neurotrophic factor (CNTF), and brain-derived neurotrophic factor (BDNF), can significantly slow retinal degeneration and cell death in animal models [14–18]. One possible source of autologous GFs is platelet-rich plasma (PRP). PRP is defined as a biological product that features platelet concentration; it is collected from centrifuged whole blood [19]. Through the activation of a reactivator (such as sodium chloride or citrate), accumulated platelets can secrete a large quantity of preparations rich in growth factors (PRGFs) via the release of intracellular α -granules [20]. PRGFs are an aggregation of cytokines that include transforming growth factors (TGF- β), interleukine-6 (IL-6), BDNF, and vascular endothelial growth factors (VEGF) [21, 22]. The strong restoring function of autologous PRP (aPRP) is based mainly on the trophic capacity of PRGFs [21].

Currently, PRP is being tested as a therapeutic option in some clinical situations, for example in orthopedics, ophthalmology, and healing therapies. Some pre-clinical and clinical trials have addressed the use of PRP and various GFs, such as

the intravitreal injection of bFGF in retinal dystrophy and the topical applications of NGF to treat glaucoma and neurotrophic keratitis [19, 23–28]. The use of PRGFs in ophthalmology has been successfully applied to ocular surface disorders, including the treatment of ocular surface syndrome [29] and flap necrosis [30] after LASIK surgery. A recent study observed that administration of platelet-derived proteins adjacent to the lacrimal gland restored lacrimal function in all patients [31]. The clinical and pre-clinical use of aPRP in ophthalmology has encouraged practitioners to use it through subtenon injection in the treatment of retinal diseases. Through the subtenon injection of PRP, the level of neurotrophic growth factors may be increased in the microenvironment around the photoreceptors, thus potentially reactivating photoreceptors that are in sleep mode. Fetal bovine serum, allogeneic serum, and umbilical cord serum have also been used as sources of growth factors, but they are heterologous products with a higher risk of allergic reactions and infectious disease transmission [32–35]. In order to avoid these issues, and because of the accessibility and relatively safe nature of aPRP, we chose to use aPRP as a source of growth factors in our study.

The purpose of this prospective open-label clinical trial was to determine whether the subtenon injection of aPRP may have beneficial effects on visual functions—such as best-corrected visual acuity (BCVA), visual field (VF), multifocal electroretinography (mfERG), and microperimetry (MP)—in RP patients with various degrees of narrowed visual fields. To our knowledge, this is the first study evaluating the effects of subtenon injections of aPRP in patients with RP.

Materials and methods

This prospective open-label clinical trial was conducted at the Department of Ophthalmology of Ankara University’s Faculty of Medicine. The study subjects all came from Turkey’s RP population. RP patients with various degrees of BCVA and narrowed VF were studied between September 2016 and February 2017. The diagnosis of RP was based on clinical history, fundus appearance, VF test, full-field ERG, and/or mfERG findings. In order to support the diagnosis in some suspected subjects, spectral domain optical coherence tomography (SD-OCT) (Spectralis, Heidelberg Engineering, Germany) was used to assess the intraretinal layers as well as the decreased thickness of the outer nuclear layer. Short-wave fundus autofluorescence (SW-FAF) (Spectralis, Heidelberg Engineering, Germany) testing was also used to see the hyperautofluorescent rings around the macular area, loss of RPE cells, and pigmentary changes in the peripheral retina in some cases [7, 36]. Dietary supplements and fish-rich Mediterranean-style diets were suspended in patients 1 month before enrolling in the study since these may interfere with visual functions.

Subjects

RP patients were included in this study if they were found to meet the following criteria:

- 18 years of age or older;
- Diagnosis of any phenotypic variation of RP, confirmed by clinical history, fundus appearance, VF, and electroretinogram;
- Experience of various degrees of VF loss;
- BCVA from light perception of up to 110 letters (equal to 1.6 decimal values) in early treatment of diabetic retinopathy study (ETDRS) chart testing (Topcon CC-100 XP, Japan);
- Mean deviation (MD) values from -33.0 to -5.0 dB with Humphrey or Octopus 900 visual field analysis (threshold 30-2, Sita Standard, Stimulus 3-white);
- Intraocular pressure (IOP) < 22 mmHg.

RP patients were excluded from the study if any of the following was found:

- The presence of cataracts or other media opacity that might affect the VF, MP, or mfERG recordings;
- The presence of glaucoma, which causes visual field and optic disc changes;
- The presence of any systemic disorder (e.g., diabetes, neurological disease, or uncontrolled systemic hypertension) that may affect visual functions;
- The habit of smoking.

Preparation and injection of autologous platelet-rich plasma

There are a variety of protocols and special kits for preparing PRP [19–22]. We used the single-spin protocol, according to which 10–15 ml of blood is drawn from the patient's antecubital vein and inserted into four 3.0 ml vacutainer tubes that contain trisodium citrate. These four tubes were placed in a centrifuge machine, and centrifugation was carried out at 2500 rpm ($580\times g$) for 8 min within a 30-min blood collection period. As a result of centrifugation, the plasma was separated in the vacutainer tubes from the remaining blood components. Three different layers formed in the tubes: red blood cells at the bottom, aPRP in the middle layer, and aPPP in the top layer. A total of 1.5 ml of the middle layer (which mainly contained platelets) was withdrawn by syringe, and it was immediately injected into the subtenon space of each eye. The preparation and injection of aPRP and aPPP were carried out by the same ophthalmologist (UA), under topical anesthesia and

sterile conditions. The subjects were asked to look inferonasally, and the 1.5-ml injection of aPRP was performed under the tenon space in the superotemporal quadrant using a 25-gauge needle. This site was preferred for injection because of its easy access and relatively wide absorption area. We injected the aPPP into the subtenon space as a placebo in the eyes used as control to exclude the mechanical effects of the subtenon injection, which may induce the release of growth factors from the injured tissue. Subtenon injections were carried out immediately after the preparation of aPRP or aPPP, with each patient undergoing injections three times with 3-week intervals between each injection. The patients were followed for three more weeks after the third injection; thus, the total duration of the study for each patient was 9 weeks. The results were obtained from a comparison of the pre-injection and final examination values.

In this preliminary prospective clinical study, the primary aim was to assess the effects of aPRP on BCVA, VF, and mfERG. However, when there was macular involvement confirmed by SD-OCT and FAF, we performed MP to evaluate the change of macular functions before and after subtenon aPRP injections. The secondary aim of the study was to evaluate whether the mechanical effects of subtenon injections, which may induce the release of growth factors from the injured tissue, have any effects on visual functions.

All patients enrolled in this study underwent a complete routine ophthalmic examination on both eyes, with the ETDRS chart used to measure visual acuity. The changes in BCVA and VF were analyzed appropriately on the patients whose BCVA values were better than 50 letters (equal to 0.1 decimal) on the ETDRS chart (Topcon CC 100 XP, Japan). For VF analysis, two types of machines were used for technical reasons (the Threshold 30-2 Humphrey VF by HFA II 750 device (Carl Zeiss Meditec AG, Germany) and the Threshold 30-2 Octopus 900 Goldmann Perimetry (Haag Streit, Switzerland)); however, each patient had all tests conducted using the same instrument and the same technician. In order to avoid mistakes during the test, practice rounds were carried out three times for each eye. These visual field practice tests were done using the same parameters as the real test to exclude learning effects. MP assessment was performed only on patients who had macular involvement, using MAIA (CenterVue, Italy).

To evaluate retinal function, mfERG (Retiscan, Roland Germany) could be performed on patients who had sufficient fixation according to ISCEV standard protocol [37]. The mfERG measures neuroretinal function (postreceptoral responses, cone mediated ON and OFF bipolar cells, and inner retinal cell contributions) in localized retinal areas [38]. The amplitude (nv/deg^2) and implicit times (ms) of the first-order

kernel mfERG responses (N1 and P1 waves) were obtained and grouped into five rings (ring 1, central 2°; ring 2, 2–5°; ring 3, 5–10°; ring 4, 10–15°; ring 5, > 15°). In all subjects, the mfERG testing protocol was started after 20 min of pre-adaptation to an ambient light environment equivalent to the mean luminance of the stimulus, at 100 cd/m². Pupils were pharmacologically (with tropicamide 1%) dilated to 8–9 mm. The cornea was anesthetized with 0.4% oxybuprocaine. The mfERGs were recorded monocularly, patching the contralateral eye, by means of a ERG-jet contact lens electrode using methylcellulose. A small gold skin ground electrode was placed at the center of the forehead after preparing the skin with abrasive gel and filling the electrode cup with electrolyte gel. Meanwhile, a skin electrode was placed at the outer canthus, to be used as a reference. mfERG was not applied to the subjects who had over ± 3.0 D refraction errors, so refractive correction was not applied. The multifocal stimulus, consisting of 61 scaled hexagons, was displayed on a high-resolution, black and white cathode ray tube (CRT) monitor with a frame rate of 75 Hz. The signal was amplified (gain 100,000) and filtered (band pass 3–300 Hz). After automatic rejection of artifacts, the first-order kernel response, K1, was examined. These parameters were obtained from five concentric annular retinal regions (rings) centered on the fovea.

Time frame

Injections were done three times, with each injection followed by a 3-week interval period. The patients were then followed for 3 weeks following the final injection. The time frames recorded were as follows:

- Before application: a period of three months prior to PRP application
- 0 (baseline): just before the first PRP injection
- 1: the time of the second PRP injection
- 2: the time of third (last) PRP injection
- 3: the time of the final examination 3 weeks after the third PRP injection

Primary outcome measure

- Visual field sensitivity (time frame: before application, 0, 1, 2, and 3)

A Humphrey or Octopus 900 visual field analyzer, threshold 30-2 modality, was used at time points of 0, 1, 2, and 3. In addition, it was used three times before application during experimentation to exclude the learning effect. The MD values, which were obtained from the baseline test and the final examination, were analyzed and compared statistically to make conclusions regarding effectiveness. Visual field

analysis could be properly performed on patients whose BCVA values were better than 50 letters in ETDRS chart testing (0.1 decimal).

Secondary outcome measures

- ETDRS visual acuity (time frame, 0, 1, 2, and 3)

Visual acuity was measured at the time points of 0, 1, 2, and 3. The visual acuity score values obtained from the baseline testing and the final examination were analyzed and compared statistically to make conclusions regarding effectiveness.

- Amplitudes of multifocal electroretinogram [Time frame: 0 and 3]

The retinal electrical responses from mfERG were measured in patients with less than ± 3.0 D refraction errors at the time points of 0 and 3. The amplitudes of each ring, obtained during baseline testing and in the final examination, were analyzed and compared statistically to make conclusions regarding effectiveness.

- Implicit times of multifocal electroretinogram (time frame, 0 and 3)

The implicit times of each ring, obtained from the baseline testing and the final examination, were analyzed and compared statistically to make conclusions regarding effectiveness.

- Microperimetry (time frame, 0 and 3)

The average threshold values were measured by 4–2 strategy at the time points of 0 and 3. The average threshold values, obtained from the baseline testing and the final examination, were analyzed and compared statistically to make conclusions regarding effectiveness.

The statistical comparisons were made mainly between the baseline and final values from the same eye. However, to exclude the mechanical effect of the injection, which could induce the release of growth factors from the injured tissue, two eyes were compared with a placebo in a small group. PPP was used as the placebo.

Eleven patients who had symmetrical bilateral-narrowed VF in both eyes were chosen (from out of the 48 RP patients) as a comparison group to assess the mechanical effect of subtenon injections. In this group, patients had one eye injected with aPRP and the other eye injected with aPPP. As in the main group, injections were carried out three times, with intervals of 3 weeks between injections. Since mfERG provides the fastest and most objective results, the eyes in this group were compared only using mfERG.

Definition of safety outcome

Intravitreal/subretinal/macular hemorrhages, vitreoretinal interface alterations, retinal tear(s)/retinal detachment (exudative, rhegmatogenous), cataract formation, intraocular pressure change from baseline (≤ 5 mmHg), intraocular inflammation, and ocular allergic reactions were considered to be serious adverse ocular events. Besides routine ophthalmic examinations, SD-OCT and SW-FAF were also used to detect and confirm the presence of complications and anatomical changes during each examination in the study period. Systemic allergic reactions and anaphylaxis were considered systemic side effects.

Statistical methods

The BCVA and parametric results for visual field, microperimetry, and mfERG were analyzed using the *t* test and the Wilcoxon test. Results were presented as mean and standard deviation. The Wilcoxon test was also used for comparison of aPRP and aPPP groups. In this study, *p* values smaller than 0.05 were considered statistically significant. A 95% confidence interval for the difference in means was used for double confirmation. Analyses were carried out with SPSS for Windows (v22; IBM Corp.; Armonk, NY, USA).

Results

In this study, 71 eyes belonging to 48 RP patients were included. The remaining 25 eyes were excluded because of the absence of light perception. Of the 48 patients, 28 were male and 20 were female; their median age was 32 years (range, 18–55 years). Forty-nine eyes belonging to 37 patients were injected with aPRP, with the remaining 11 patients having one eye was injected with aPRP and the other injected with aPPP.

We did not encounter any serious adverse events related to aPRP preparation or subtenon injection. We also did not encounter any ophthalmic or systemic side effects due to subtenon-injected aPRP itself in any of the examination modalities mentioned in the study. Not all tests could be performed for every patient due to reasons such as the level of VA, ocular status, macular involvement, patient in compliance, or technical and socio-economic issues. Table 1 displays the tests that could be done, along with the number of eyes, patients, and the time frame for each test.

The BCVA of the 71 studied eyes ranged from light perception of up to 110 letters (1.6 decimals) on the ETDRS chart. Since BCVA values better than 50 letters (0.1 decimal) on the ETDRS chart were required in order to evaluate both changes to VA and VF after the injections, 48 out of the 71 eyes (67.6%) were used for these purposes. These data are presented in Table 2. The BCVA values in the ETDRS chart improved by 11.6 letters (from 70 to 81.6 letters) in 19 of the 48 eyes following aPRP application; this result, however, was not statistically significant ($p = 0.056$) (Tables 2 and 3).

Thirty-eight patients (79%) stated subjectively that, relative to their pre-treatment vision, colors seemed brighter and clearer, their sight of the surrounding environment seemed better, and the time it took them to adapt to darkness seemed shorter. They also noted a decrease in photopsia and glare.

Visual field mean values were obtained in 48 eyes belonging to the 40 subjects on whom a visual field test could be performed. Statistically significant VF improvement was detected over the 9-week study period when comparisons were made before and after the injections of aPRP ($p = 0.0001$) (Table 4; Figs. 1, 2, and 3).

In the mfERG results of 33 eyes (of 33 subjects), P1 amplitudes improved in ring 1 from 24.4 to 38.5 nv/deg^2 ($p = 0.001$), in ring 2 from 6.7 to 9.3 nv/deg^2 ($p = 0.0301$), and in ring 3 from 3.5 to 4.5 nv/deg^2 ($p = 0.0329$). mfERG P1 implicit times improved in ring 1 from 40.0 to 34.4 ms ($p =$

Table 1 The tests that could be done on 71 eyes (48 RP patients)

Tests done	No. of eyes	No. of patients	Time frame*
BCVA (ETDRS ≥ 50 letters)	48	40	0, 1, 2, 3
Visual field	48	40	BA, 0, 1, 2, 3
mfERG	33	26	0, 3
Microperimetry	23	18	0, 3

*Time frame: injections were done three times, with a 3-week interval between injections, and patients were then followed for three more weeks

- Before application (BA): the period of 3 months prior to PRP application
- 0 (baseline): just before application of the first PRP injection
- 1: the time of the second PRP injection
- 2: the time of the third (last) PRP injection
- 3: final examination 3 weeks after the third PRP injection

Table 2 Changes in BCVA and visual field MD values after aPRP injections in 48 eyes (40 patients)

No.	Age	Gender	Eye	BCVA (ETDRS letters)		BCVA (decimal)		Visual field MD values	
				Before PRP	Last exam after PRP	Before	After	Before PRP	Last exam after PRP
1	32	M	R	105	105	1.25	1.25	14.6	5.0
	32	M	L	105	105	1.25	1.25	12.8	3.1
2	18	M	R	110	110	1.6	1.6	22.39	14.44
	18	M	L	110	110	1.6	1.6	22.02	16.67
3	45	F	R	95	95	0.8	0.8	21.72	10.33
	45	F	L	95	95	0.8	0.8	23.97	18.52
4	44	M	R	100	100	1.0	1.0	25.68	23.98
5	26	F	L	105	105	1.25	1.25	21.95	20.17
6	22	M	R	54	54	0.12	0.12	27.23	23.47
7	22	F	L	89	89	0.6	0.6	20.34	17.96
	22	F	R	85	89	0.5	0.6	18.48	16.07
8	32	F	L	80	80	0.4	0.4	32.57	27.10
	32	F	R	50	85	0.1	0.5	29.90	27.10
9	30	M	L	60	60	0.16	0.16	11.94	8.97
10	25	M	L	70	75	0.25	0.32	18.95	16.40
	25	M	R	75	80	0.32	0.4	18.54	15.93
11	29	M	R	54	80	0.12	0.4	33.43	30.16
	29	M	L	75	80	0.32	0.4	33.70	30.26
12	26	M	L	50	50	0.1	0.1	33.55	30.45
13	23	F	R	54	65	0.12	0.2	31.54	29.78
14	40	M	L	80	89	0.4	0.6	29.50	29.52
15	38	F	R	60	40	0.16	0.063	33.07	32.61
	38	F	L	60	85	0.16	0.5	33.07	32.61
16	28	M	R	60	65	0.16	0.2	32.56	31.03
17	55	M	R	70	75	0.25	0.32	31.85	31.62
18	53	F	R	92	100	0.7	1.0	31.44	31.18
19	25	F	L	70	75	0.25	0.32	28.64	28.27
20	40	M	R	50	85	0.1	0.5	32.90	32.14
21	30	F	R	50	60	0.1	0.16	32.72	32.23
22	50	F	R	75	80	0.32	0.4	30.88	29.29
23	26	M	R	80	89	0.4	0.6	22.86	22.07
24	43	M	R	80	89	0.4	0.6	26.38	26.42
25	24	M	L	105	105	1.25	1.25	20.38	19.47
26	32	M	R	100	100	1.0	1.0	26.94	26.09
27	23	M	R	100	105	1.0	1.25	26.30	24.99
28	26	M	R	110	110	1.6	1.6	11.82	10.61
29	21	M	L	110	110	1.6	1.6	14.40	13.01
30	28	M	L	100	100	1.0	1.0	31.58	30.63
31	48	F	R	100	100	1.0	1.0	28.76	28.44
32	30	M	R	105	105	1.25	1.25	22.30	22.50
33	43	F	R	110	110	1.6	1.6	13.80	13.82
34	44	F	R	90	90	0.63	0.63	27.48	27.63
35	34	M	R	89	89	0.6	0.6	29.35	28.42
36	27	F	R	85	85	0.5	0.5	30.90	30.82
37	34	F	R	80	80	0.4	0.4	27.52	26.10
38	37	M	R	80	80	0.4	0.4	27.44	26.35
39	47	F	R	65	65	0.2	0.2	32.71	32.49
40	45	M	L	50	50	0.1	0.1	5.24	3.98

BCVA, best-corrected visual acuity; ETDRS, early treatment of diabetic retinopathy study charts (letters point); MD, mean deviation

Table 3 Comparison of BCVA at baseline and final examination

BCVA	N (eye)	Mean (letters)	Standard deviation	<i>t</i> value	<i>p</i> value
Baseline exam before aPRP	48	81.81	20.16	1.12	0.056*
Final exam after 3rd aPRP	48	86.00	18.03		

BCVA, best-corrected visual acuity; aPRP, autologous platelet-rich plasma

*Statistically non-significant

Table 4 Comparison of visual field mean deviation (dB) at the baseline and final examination

Visual field mean deviation (dB)	N (eye)	Mean (dB)	Standard deviation	%95 CI L	%95 CI U	t value	p value
Baseline exam before aPRP	48	-25.37	7.21	1.46	3.03	5.77	0.0001*
Final exam after aPRP	48	-23.12	8.43				

aPRP, autologous platelet-rich plasma; %95 CI L and U, 95% confidence interval of the difference (mean)

*Statistically significant

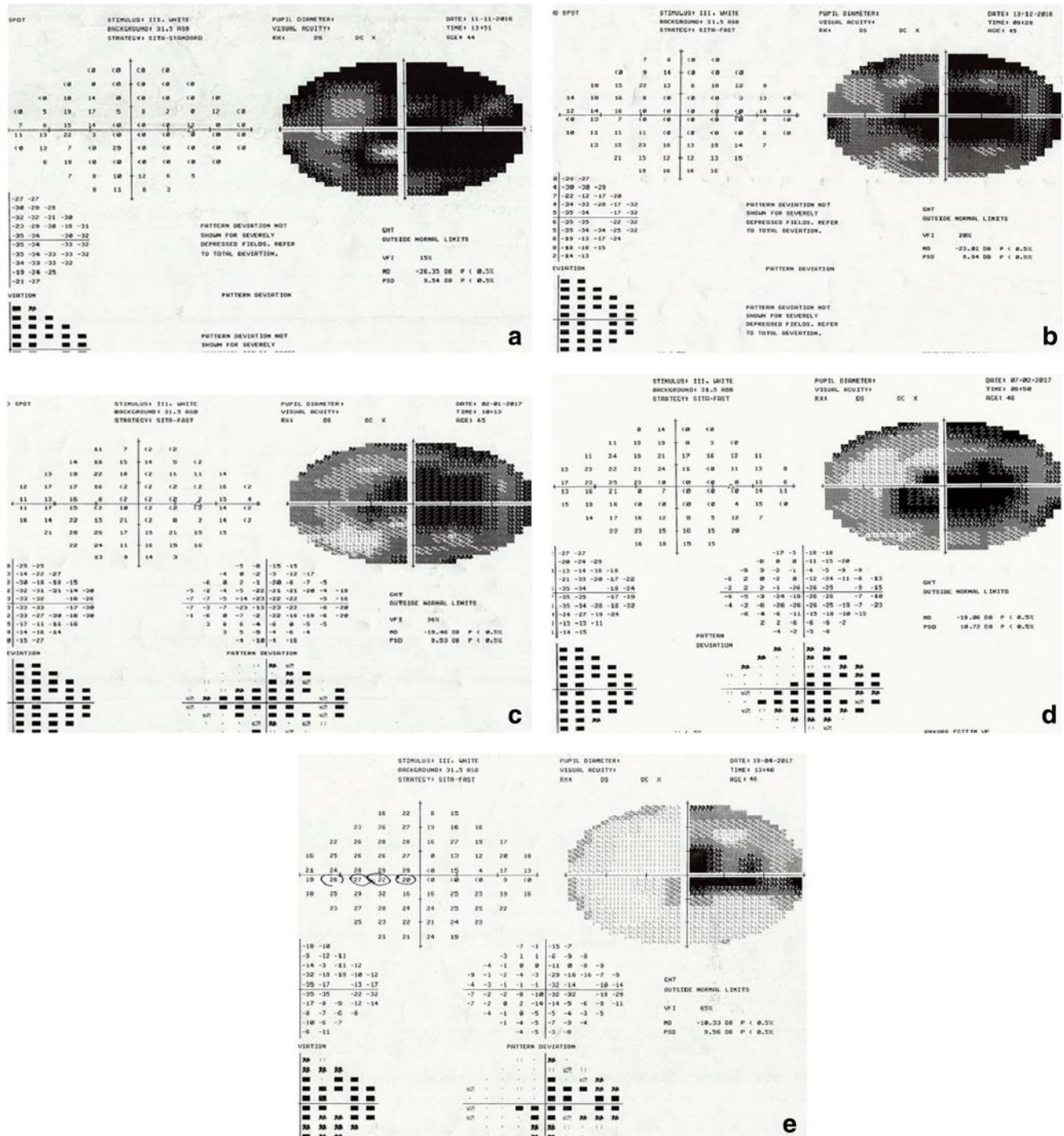


Fig. 1 a–e Visual field changes after aPRP injections (Table 2, patient no. 3: right eye): a before aPRP application, b after 1st aPRP application, c after 2nd aPRP application, d after 3rd aPRP application, and e at the final exam

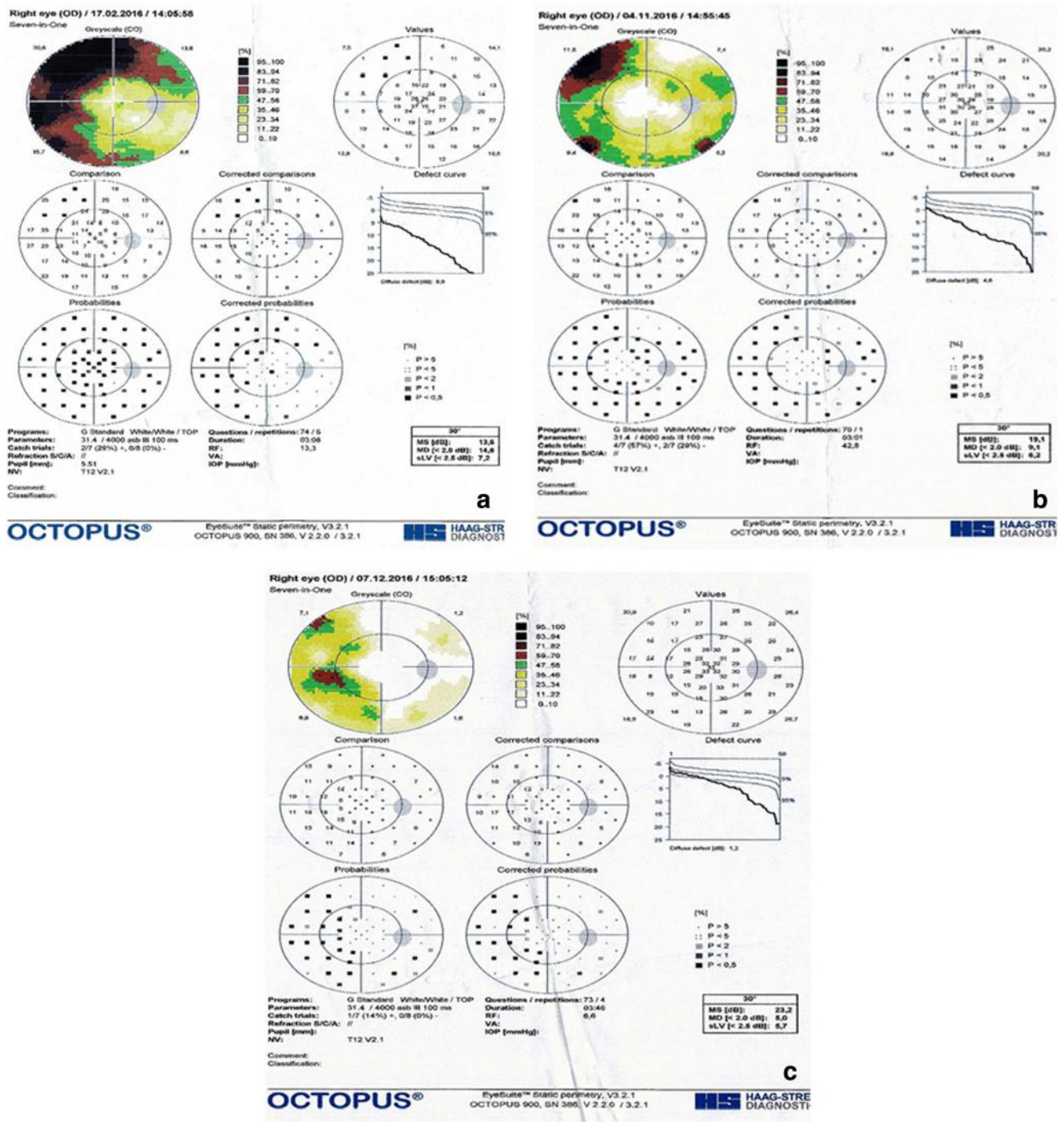


Fig. 2 a–c Visual field changes after aPRP injections (Table 2, patient no. 1: right eye): **a** before aPRP application, **b** after 2nd aPRP application, and **c** at the final exam

0.01), in ring 2 from 42.5 to 33.2 ms ($p = 0.01$), and in ring 3 from 42.1 to 37.9 ms ($p = 0.04$). mfERG N1 amplitudes improved in ring 1 from 0.18 to 0.25 nv/deg^2 ($p = 0.011$) and in ring 2 from 0.05 to 0.08 nv/deg^2 ($p = 0.014$). mfERG N1 implicit time also improved in ring 1 from 18.9 to 16.2 ms ($p = 0.040$) and in ring 2 from 20.9 to 15.5 ms ($p = 0.002$) (Tables 5 and 6; Figs. 4 and 5).

Microperimetry tests were performed for RP cases with macular involvement (i.e., 23 eyes among 18 patients). The average microperimetry threshold dB values showed statistically significant improvement following aPRP injections ($p = 0.0001$) (Table 7; Fig. 6).

In the comparison group (i.e., the 11 patients who had one eye injected with aPRP and the other eye injected with aPPP),

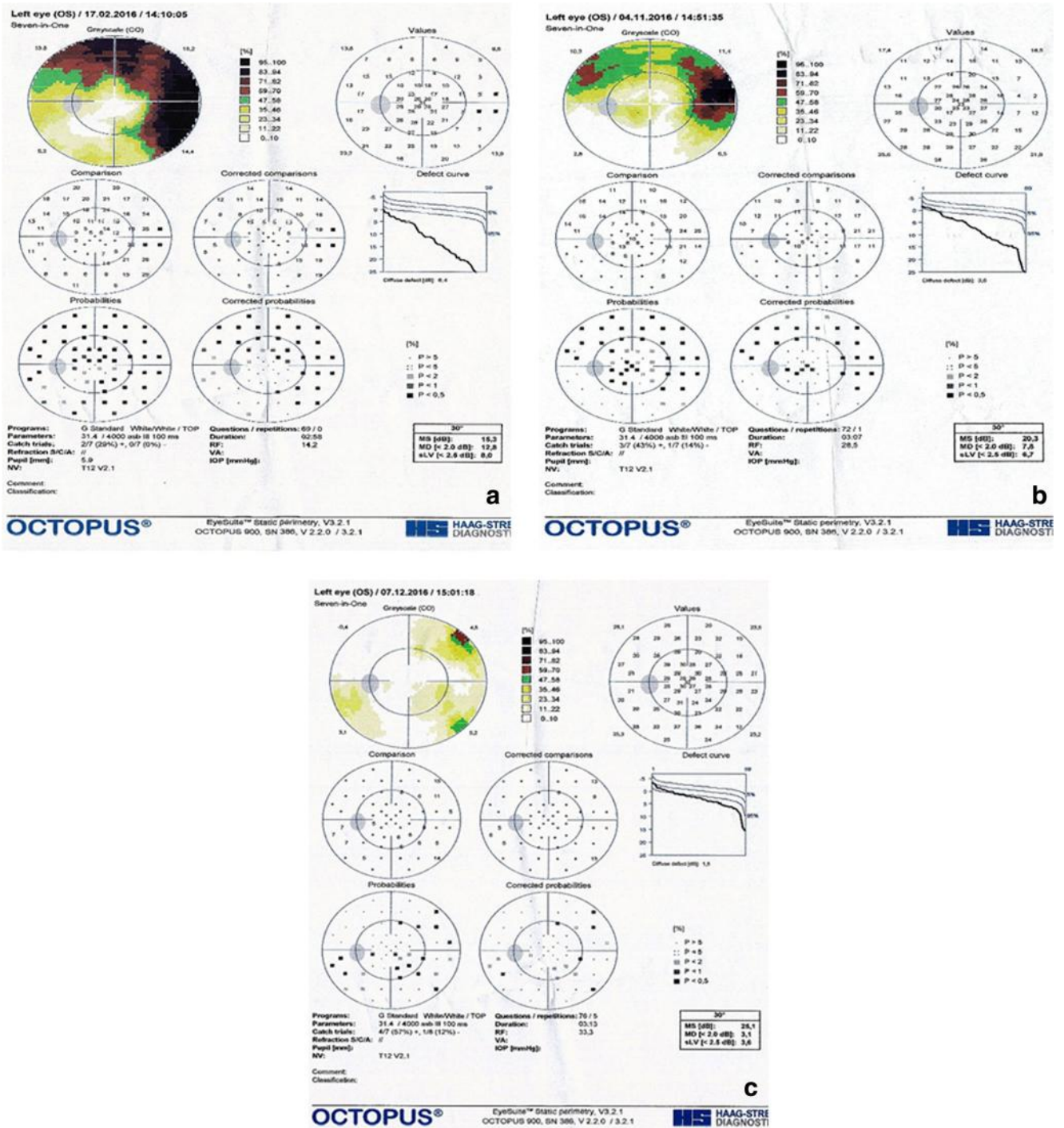


Fig. 3 a–c Visual field changes after aPRP injections (Table 2, patient no. 1: left eye): **a** before aPRP application, **b** after 2nd aPRP application, and **c** at the final exam

the P1 amplitudes and P1 implicit times of the eyes injected with aPRP showed statistically significant improvement in rings 1, 3, and 4, whereas the eyes injected with aPPP did not show any improvement (Tables 8 and 9).

Discussion

RP is an outer retinal degenerative disease that destroys photoreceptors while leaving a significant percentage of the inner retinal cells intact and functional [39–42]. It is not well

Table 5 Comparison of mfERG parameters (P1) at the baseline and final examination

Ring	P1 amplitude (nv/deg ²)				P1 implicit time (ms)							
	Baseline		Final		<i>p</i> value	%95 CI (L, U)	Baseline		Final		<i>p</i> value	95% CI (L, U)
	<i>X</i> ± SD		<i>X</i> ± SD				<i>X</i> ± SD		<i>X</i> ± SD			
1	24.42	19.02	38.51	20.75	0.0001	− 19.44, − 8.73)	40.06	7.21	34.43	6.71	0.01	12.02, 6.59
2	6.79	8.28	9.35	7.24	0.0301	− 4.86, − 0.26)	42.58	11.07	33.26	8.48	0.01	13.26, 7.12
3	3.52	2.42	4.58	2.89	0.0329	− 2.03, − 0.09	42.16	9.96	37.94	9.16	0.04	9.24, 8.73
4	2.75	2.89	3.03	2.37	0.4347	− 1.01, − 0.44	36.76	11.24	40.15	10.07	0.10	− 6.28, − 2.11
5	1.89	2.35	2.01	1.82	0.6291	− 0.61, − 0.37	40.44	9.20	41.38	9.37	0.55	− 0.45, − 0.28

X, mean values obtained from 33 eyes (26 patients); SD, standard deviation; %95 CI L and U, 95% confidence interval of the difference (mean)

*Significant *p* values and related data (i.e., coefficient and standard error) are set in italics

understood why some photoreceptors die while others do not, and this question has spurred fascinating and important research in which gene and drug therapies have rendered preliminarily positive results. Retina remodeling and structure studies have shown that before photoreceptor cell death, stress on photoreceptors can induce ectopic synaptogenesis and Müller cell hypertrophy at about 19° of the central visual field. There is also an increase in growth factor synthesis by the paracrine effect of Müller cells. These compensatory changes may explain why the central visual field is affected in late stages [36, 43–50].

In our study, results regarding the mfERG P1 amplitude and the implicit time of retinal electrical activity improved following aPRP injections in rings 1, 2, and 3. The aforementioned compensatory changes developing in the macula may explain why our study saw significant improvement in the inner rings of the mfERG. Since photoreceptors in the area surrounding the macula may not be reactivated by aPRP injections, a non-significant improvement in the outer rings of the mfERG was detected. aPRP injections did not lead to a significant change in visual acuity over the study period. Since central vision is generally preserved in RP, a measurement of

visual acuity is not the best way to evaluate the functional results of the treatment. Due to the limited healthy central retinal area, visual acuity might not change even in patients with advanced RP.

The full-field ERG allows a recording of the electrical responses originating from the entire retina when stimulated with a full-field light source. In contrast, the multifocal ERG (mfERG) allows an assessment of a “map” of electrical activity [38, 51–53]. The mfERG has been applied to various retinal disorders, and a standard has become available (see also www.isceev.org). As the disease progresses, the waveforms may no longer be detected by full-field ERG, whereas some residual VF can still be measured. In such cases, localized responses may still be obtained using the mfERG [38]. The clinical full-field ERG tests both rod and cone function, while the clinical mfERG tests only cone function. However, mfERG gives more sensitive results than full-field ERG in detecting changes, so we used mfERG in our study.

Cells can enter a dormant state when faced with unfavorable conditions. This transition is required for cellular survival under conditions of starvation. However, the way in which

Table 6 Comparison of mfERG parameters (N1) at the baseline and final examination

Rings	N1 amplitude (nv/deg ²)				N1 implicit time (ms)							
	Baseline		Final		<i>p</i> value	%95 CI (L, U)	Baseline		Final		<i>p</i> value	%95 CI (L-U)
	<i>X</i>	SD	<i>X</i>	SD			<i>X</i>	SD	<i>X</i>	SD		
1	0.18	0.14	0.25	0.14	0.011*	− 0.132, − 0.029	18.95	5.93	16.23	4.39	0.040*	− 0.238, 5683
2	0.05	0.05	0.08	0.07	0.014*	− 0.055, − 0.006	20.91	6.83	15.59	5.15	0.002*	2104, 8543
3	0.05	0.04	0.07	0.05	0.128	− 0.037, 0.005	16.91	6.44	18.68	7.40	0.098	− 3893, 0.348
4	0.06	0.06	0.07	0.06	0.297	− 0.292, 0.009	19.04	6.27	17.20	6.43	0.185	− 0.924, 4.59
5	0.06	0.05	0.06	0.05	0.793	− 0.021, 0.016	19.23	6.34	19.97	6.70	0.625	− 4237, 2851

X, mean values obtained from 33 eyes (26 patients); SD, standard deviation; %95 CI (L and U), 95% confidence interval of the difference (mean)

*Significant *p* value and related data (i.e., coefficient and standard error) are set in italics

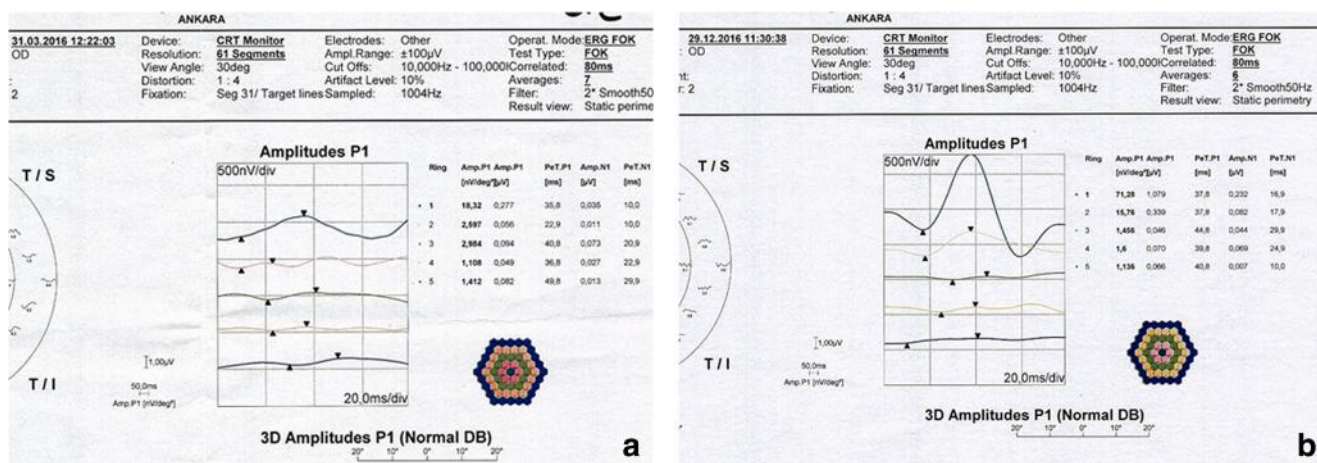


Fig. 4 a, b Changes of mfERG parameters (P1-N1 amplitudes and implicit times) before and after aPRP injections (Table 2, patient no. 8: right eye): a before aPRP application and b at the final exam

cells enter into and recover from this state remains poorly understood. Some proposed mechanisms have been found to trigger a transition of the cytoplasm to a solid-like state with increased mechanical stability. These findings have broad implications for understanding alternative physiological states, such as quiescence and dormancy, and create a new view of the cytoplasm as an adaptable fluid that can reversibly transition into a protective solid-like state [8–13, 54–56].

Genetic mutations lead to defects in the synthesis of rhodopsin or in growth factors required for photoreceptor metabolism from the retinal pigment epithelium. The lack of rhodopsin synthesis can lead to the opening of calcium ion channels and may induce apoptosis in photoreceptors [1, 57, 58]. One of the main causes of photoreceptor degeneration is apoptosis. Due to the growth factors released by Müller cells, photoreceptors do not undergo apoptosis immediately. First, photoreceptors slow down their metabolic activities. If the growth factor deficiency continues for a long time, the photoreceptors will undergo apoptosis [9–13, 36, 54].

Most retinitis pigmentosa mutations arise in rod photoreceptor genes, leading to diminished peripheral and nighttime vision. Glucose becomes sequestered in the retinal pigment epithelium, and thus is not transported to photoreceptors. The resulting starvation for glucose metabolites impairs synthesis of cone visual pigment-rich outer segments, and then their mitochondrial-rich inner segments dissociate. Loss of these functional structures diminishes cone-dependent high-resolution central vision, which is utilized for most daily tasks [9]. Circulating glucose from the choroid is transported to the retinal pigment epithelium and then into the subretinal space for uptake by a Glut1 complex on photoreceptor mitochondrial-rich inner segments [71, 72]. Recent studies suggest the thioredoxin family member rod-derived cone viability factor (RdCVF) can promote glucose uptake into cones in cell culture [72], and its overexpression in the subretinal space is neuroprotective and can delay transition to cone dormancy in rodent RP models [12, 73]. Glucose induction of thioredoxin-interacting protein (Txnip), the most highly

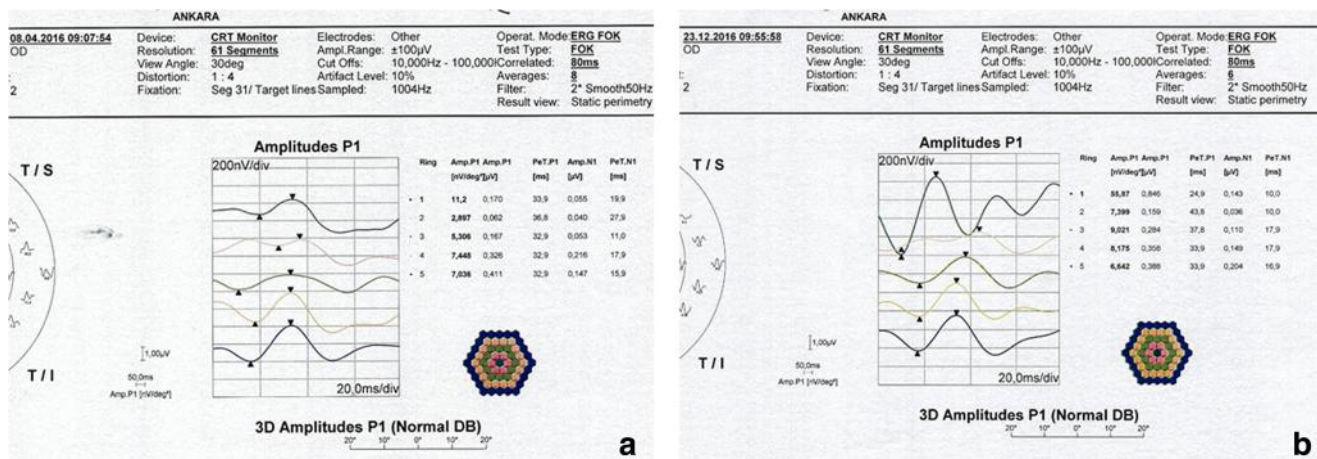


Fig. 5 a, b Changes of mfERG parameters (P1-N1 amplitudes and implicit times) before and after aPRP injections (Table 2, patient no. 11): a before aPRP application and b at the final exam

Table 7 Comparison of microperimetry threshold (dB) values at the baseline and final examination

Microperimetry threshold dB	N (eye)	Mean	Standard deviation	%95 CI L	%95 CI U	t value	p value
Baseline exam before aPRP	23	15.04	6.28	-1.99	-0.88	-5.38	0.0001*
Final exam after aPRP	23	16.48	6.51				

*Statistically significant

glucose-inducible gene identified to date, regulates Akt activity to divert cells toward aerobic glucose metabolism and fatty acid synthesis [74–76]. Circadian rhythms are self-sustained, approximately 24-h rhythms of physiology and behavior. These rhythms are entrained to an exactly 24-h period by the daily light-dark cycle. Remarkably, mice lacking all rod and cone photoreceptors still demonstrate photic entrainment, an effect mediated by intrinsically photosensitive retinal ganglion cells (ipRGCs). These cells utilize melanopsin (OPN4) as their photopigment. Distinct from the ciliary rod and cone opsins, melanopsin appears to function as a stable photopigment utilizing sequential photon absorption for its photocycle; this photocycle, in turn, confers properties on ipRGCs such as sustained signaling and resistance from photic bleaching critical for an irradiance detection system. The retina itself also functions as a circadian pacemaker that can be autonomously entrained to light-dark cycles [77]. The diurnal protein rhythms are specifically identified as enzymes involved in glucose metabolism, Krebs cycle, and mitochondrial enzymes. Lacking the circadian clock of the photoreceptors, might be caused by a circadian clock in other retinal cell types or a direct light input to the retina [78]. Based on this information, we think that glucose metabolism is activated by the aPRP content. This information may also explain the deterioration of mfERG results in the aPPP group.

Apoptosis is recognized as a common form of cell death and a mechanism of cell clearance in many physiological

situations where cell deletion is required. Peptide growth factors, initially characterized as stimulators of cell proliferation, have now been shown to inhibit death in many cell types. The deprivation of growth factors leads to the induction of apoptosis [55]. Following growth factor withdrawal, cells activate self-autophagy, undergo progressive atrophy, and ultimately succumb to death. It is thought that these effects derive from the loss of the ability to take in sufficient nutrients to maintain cellular bioenergetics. Thus, to maintain cell viability, growth factor signal transduction is needed to direct the use of sufficient exogenous nutrients [56]. Neurotrophic factors comprise a family of growth factors that play key roles in the development and survival of neurons. The potency of neurotrophic factors in promoting neural survival has raised much hope for their therapeutic use in treating neurodegenerative diseases [28, 59–68]. In treating retinal degenerative disease, growth factor supply can be used to improve the status of retinal photoreceptors [16, 63].

This line of thinking is supported by this prospective clinical trial, as the preliminary results featured improvements in the mean sensitivity of the visual field, microperimetry, and mfERG values. Significant improvements in these values were detected after the first injection, and the values gradually increased after each aPRP injection over the study period. We believe that the death of the remaining photoreceptors can be slowed or mitigated through the use of PRP injections.

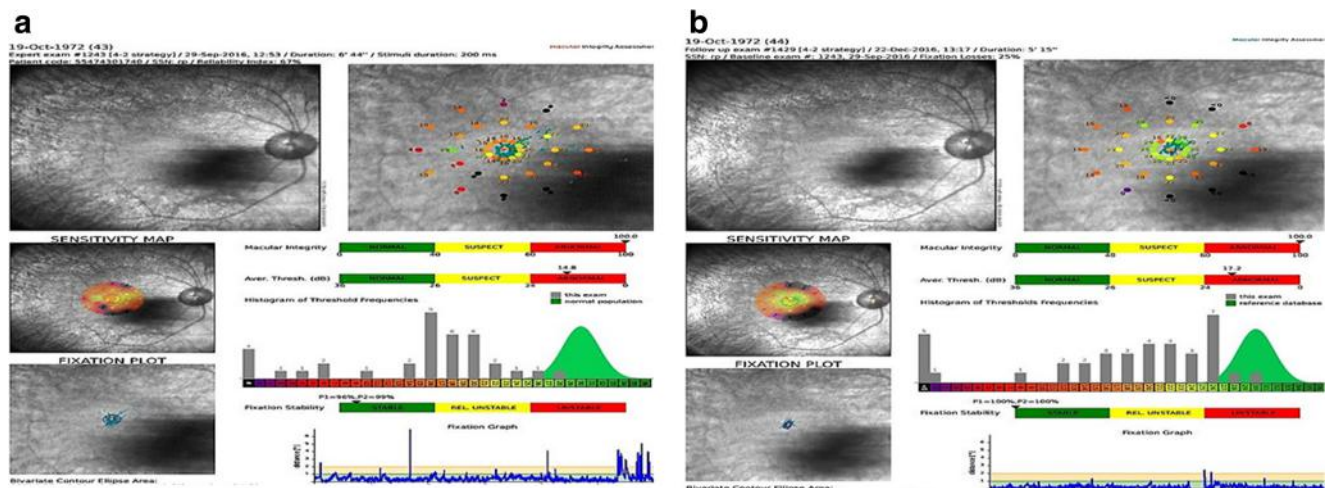


Fig. 6 **a, b** Changes of microperimetry and threshold dB values before and after aPRP injections (Table 2, patient no. 20: right eye): **a** before aPRP application and **b** at the final exam

Table 8 Comparison of mfERG P1 amplitudes between aPRP and aPPP injected eyes

Ring	P1 amplitude (nv/deg ²) (aPRP)					P1 amplitude (nv/deg ²) (aPPP)				
	Baseline		Final		<i>p</i> value	Baseline		Final		<i>p</i> value
	<i>X</i>	± Std Err	<i>X</i>	± Std Err		<i>X</i>	± Std Err	<i>X</i>	± Std Err	
1	<i>26.41</i>	<i>18.89</i>	<i>48.63</i>	<i>25.56</i>	<i>0.0009</i>	40.33	35.01	31.19	27.51	0.21322
2	6.73	7.93	11.31	5.91	0.0504	10.76	9.67	7.60	4.86	0.10951
3	<i>2.84</i>	<i>2.01</i>	<i>3.63</i>	<i>2.38</i>	<i>0.0374</i>	5.40	2.18	3.22	2.58	0.62601
4	<i>1.71</i>	<i>1.00</i>	<i>2.46</i>	<i>1.37</i>	<i>0.0100</i>	1.89	0.79	1.58	0.91	0.50702
5	1.15	0.55	1.44	0.73	0.0520	1.34	0.80	0.69	0.36	0.67002

Significant *p* value and related data (i.e., coefficient and standard error) are in set in italics

X, mean values obtained from 11 eyes; Std Err, standard error

This study used aPRP as its source of autologous growth factors. The aPRP was prepared from autologous blood by a venous puncture, a procedure found to prevent morbid infection and immunological rejection [19–22]. Platelets secrete stored intercellular mediators and cytokines (such as interleukine-6 and other inflammatory cytokines) from the cytoplasmic pool, and they release α -granule content (such as TGF- β , BDNF, and VEGF) after aggregation [20]. These secretions are intense in the first hour after injury, and platelets continue to synthesize more cytokines and growth factors from their mRNA reserves for at least another 7 days. More than 800 different proteins are secreted into the surrounding media, and they have a paracrine effect on different cell types. Cell proliferation, angiogenesis, and cell migration are stimulated, resulting in tissue regeneration [19–21]. There are many advantages inherent in applying PRP in treating neurodegenerative diseases. PRGFs provide a high level of natural recombination and contain a variety of growth factors that offer multiple neurotrophic effects [28]. Most cytokines in PRGFs are capable of inducing many types of stem cell proliferation and of differentiating into neurons and glia [19–21].

Pharmacodynamic studies have shown the ability of NGF eye drops to target the optic nerve and brain [69]. Topical administration of BDNF in the form of eye drops have led to recovered visual function and increased the number of ganglion cells in mice. Although several studies have demonstrated that neurotrophins such as NGF and BDNF can be delivered to the retina and optic nerve via eye drops, the effectiveness of this mode of neurotrophin delivery is still being validated [70]. With the subtenon injection of aPRP, the level of neurotrophic growth factors can be increased in the microenvironment around the photoreceptors, possibly leading the growth factors to diffuse into the choroid retina and thus reactivating the photoreceptors that are in sleep mode. As such, this treatment can contribute to improved visual functions, as demonstrated in our preliminary study. The literature states that the duration of the effect of aPRP ranges from 6 months to 2 years [19–21, 25]; further research is needed to determine the duration of viability and the optimal frequency of aPRP reinjections.

This clinical trial has several limitations. First, neither the patient groups nor the tests conducted were homogeneous. Thus, it was not possible to correlate the results of the mfERG with VA and VF. Second, a study of genetics and its

Table 9 Comparison of mfERG P1 implicit times between aPRP and aPPP injected eyes

Ring	P1 implicit time (ms) (aPRP)					P1 implicit time (ms) (aPPP)				
	Baseline		Final		<i>p</i> value	Baseline		Final		<i>p</i> value
	<i>X</i>	± Std Err	<i>X</i>	± Std Err		<i>X</i>	± Std Err	<i>X</i>	± Std Err	
1	<i>41.39</i>	<i>4.22</i>	<i>34.99</i>	<i>7.08</i>	<i>0.01</i>	33.89	6.81	43.66	6.81	0.20
2	<i>42.14</i>	<i>10.12</i>	<i>33.23</i>	<i>13.03</i>	<i>0.01</i>	32.33	9.05	40.03	9.69	0.12
3	<i>42.30</i>	<i>10.09</i>	<i>39.16</i>	<i>9.35</i>	<i>0.04</i>	35.79	8.71	43.26	7.39	0.12
4	37.89	9.24	39.66	9.41	0.32	35.22	7.71	44.00	5.61	0.16
5	36.45	9.75	45.24	5.91	0.16	34.43	10.23	40.50	6.65	0.16

Significant *p* value and related data (i.e., coefficient and standard error) are in set in italics

X, mean values obtained from 11 eyes; Std Err, standard error

correlation with various functional parameters fall outside of the focus of this study. The lack of genetic analysis makes it difficult to understand the response of aPRP effects to genetic mutations. In addition, the lack of genetic testing makes it difficult to distinguish types of RP from other retinal dystrophies. Third, it is not known exactly how long the aPRP effects will last; the effect and its duration may be different for each patient. Since the amount of growth factors in each injection remains unknown, the ideal time frame between injections and the amount that should be injected remain subject to investigation. Long-term research is needed to determine at what intervals the aPRP application will be required. There was insufficient information about the differences in visual function results between cases in which one eye was treated with aPRP application and cases in which aPRP was applied to both eyes. The clinical full-field ERG tests both rod and cone function, while the clinical mfERG tests only cone function. Thus, the results presented in this current report apply only to changes in cone photoreceptor function and, because of the lack of a clinical full-field ERG testing, the effects of the treatment on the rod photoreceptor function remain unknown. This is an important limitation of this study. All these limitations form the basis for separate future research topics.

Conclusion

RP is currently an incurable public health problem, with no proven therapies thus far. The preliminary results of this study are encouraging as they show that the subtenon injection of aPRP has a favorable effect on VA, VF, mfERG, and MP results; however, it apparently has no beneficial effect on visual acuity. This approach promises to be economical, relatively safe, and easily undertaken. The subtenon injection of aPRP seems to be a therapeutic option that might lead to positive results in the vision of RP patients. Long-term adverse effects are still unknown. There have not been any serious adverse events and any ophthalmic or systemic side effects for 1 year follow-up. Further studies with long-term follow-up are needed to determine the duration of efficacy and the frequency of application.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee (Ethics Committee of the Ankara University Faculty of Medicine (12-596-16/27.06.2016) and Review Board of the Drug and Medical Device Department, within the Turkish Ministry of Health and in accordance with Turkish law (93189304-514.04.01-90670/19.07.2016)) and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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

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