



Retinal function after intravitreal injection of autologous bone marrow-derived mesenchymal stromal cells in advanced glaucoma

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Abstract

Purpose To report electroretinographic (ERG) findings in advanced glaucoma treated with a single intravitreal injection of bone marrow-derived mesenchymal stem cells (MSCs).

Methods Intravitreal injection of autologous MSCs (1×10^6 cells) was performed in 2 eyes from 2 patients with open-angle glaucoma in advanced stage of optic neuropathy (ClinicalTrials.gov, NCT02330978, 01.05.2015): cup/disk ratio worse than 0.9, visual field mean deviation index lower than -15 dB, visual acuity of light perception, but controlled intraocular pressure. ERG tests were recorded at baseline and week 1, 4 and 48 after injection, using DTL electrodes following the ISCEV standard: After dark adaptation, ERG was elicited using white flashes of 0.01 cd.s/m² and 3.0 cd.s/m², followed by 10-min light adaptation (30 cd/m²) and stimuli of 3.0 cd.s/m² and 30 Hz flicker.

Results Patients did not show improvement on visual acuity or visual field after treatment. At baseline, ERG responses showed typical findings for advanced glaucoma, with a- and b-wave amplitude and latency within normative range, but reduced photopic negative responses. No noteworthy changes were observed on ERG responses for both cases up to 1 week after treatment, but at day 15, one patient showed retinal detachment with proliferative vitreoretinopathy and was removed from the trial. The other patient kept ERG responses stable throughout study period.

Conclusion Although no ERG response changes were observed after MSCs injection in one case, the complication observed on the second one, along with the lack of visual function improvement, warrants further studies involving modified MSCs to treat ocular disorders, including glaucoma.

Trial registration: ClinicalTrials.gov, NCT02330978- missed in pdf

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Introduction

There is no definitive treatment to either prevent or restore retinal ganglion cells damage responsible for the permanent loss of vision in glaucoma. Recently,

in vitro studies suggest that mesenchymal stem cells (MSCs) might induce cell-mediated retinal ganglion cell neuroprotection [1], and several reports indicate the potential of MSCs as a regenerative therapy for glaucoma [2, 3].

There are mainly two approaches under investigation for MSCs in glaucoma: implanting the cells into the ocular anterior chamber, targeting regeneration of trabecular meshwork [4] or as a neuroprotective cellular therapy [3, 5, 6]. Although viable MSCs have been identified up to 30 days after intravitreal injections [7], there is no report on the use of MSCs in glaucomatous human eyes as a neuroprotective therapeutic option.

This report describes, for the first time, the retinal function outcomes after bone marrow-derived MSCs injection in two patients with advanced glaucoma during a 1-year follow-up period.

Methods

We conducted a clinical trial (ClinicalTrials.gov, NCT02330978) at the Ribeirão Preto Medical School, University of São Paulo (USP, Brazil), on safety of the use of autologous, bone marrow-derived MSCs for treating selected cases of advanced primary open-angle glaucoma (POAG).

The study was approved by the local ethical committee (process number 14151/2010), and two blinded adults - with baseline visual acuity of light perception on their right eyes (OD) due exclusively to open-angle glaucoma - were included and signed the informed consent. The norms of the Declaration of Helsinki and the International Conference on Standardization Note for Guidance on Good Clinical Practice (ICH, Topic E6, 1995) were followed accordingly.

Patients (both male; 53 and 75 years old) showed bilateral cup/disk ratio worse than 0.9, visual field (VF) mean deviation index lower than -15 dB, but controlled intraocular pressure (IOP), and no systemic comorbidities. They showed baseline visual acuity of hand motion on their left eyes (OS). All eyes were pseudophakic.

A single intravitreal injection of 0.1 ml of a suspension containing 1×10^6 autologous bone marrow MSCs (prepared at the Blood Center of Ribeirão Preto - USP) was performed in the superior temporal region of the worst eyes (OD). According to the

approved protocol, bone marrow cells were aspirated from the patient's iliac crest, under anesthesia, separated, and cultured.

Follow-up visits were proposed at six time points: 1 and 2 weeks, 1, 3, 6, and 12 months after baseline. In all visits, participants underwent a comprehensive ophthalmic examination, including visual acuity, slit-lamp biomicroscopy, IOP measurement (Goldmann tonometry), dilated fundoscopic examination and fundus imaging, standard automated perimetry with the 24-2 Swedish interactive threshold algorithm (Carl Zeiss Meditec, Inc., Dublin, California, USA), and spectral-domain optical coherence tomography (sdOCT) with circumpapillary RNFL thickness protocol (Spectralis, Heidelberg Engineering, Heidelberg, Germany). Electroretinographic tests (ERG) were recorded at baseline and week 1 and 4, and at 12 months after injection, using DTL electrodes following the ISCEV standard [8]: After dark adaptation (25 min), ERGs were elicited using white flashes of 0.01 cd.s/m² and 3.0 cd.s/m² for rod and combined response, respectively, followed by 10-min light adaptation (30 cd/m²) and stimuli of 3.0 cd.s/m² (cone) and 30 Hz flicker.

Results

Patients did not show improvement on either visual acuity (light perception) or VF sensitivities after treatment, and also, they did not refer changes on any subjective aspect of their visual perception in the treated eye.

The slit-lamp examination did not show signs of eye inflammatory reaction: Neither anterior chamber cells nor corneal changes were noticed. No remarkable change in IOP and sdOCT measures was noted during the follow-up period (Table 1). Fundus examination presented no vitreous or retinal abnormalities up to 15-day follow-up compared to baseline.

At baseline, dark- and light-adapted ERG responses showed typical findings for advanced glaucoma, with a- and b-wave amplitude and latency within the normative range, but reduced photopic negative responses (Fig. 1). No noteworthy changes were observed on ERG responses for both cases up to 7 days after treatment, but on day 14, one patient showed an inferior retinal detachment with proliferative vitreoretinopathy (Fig. 2). The other patient kept

Table 1 Clinical parameters of eyes underwent the MSC intravitreal injection, according to the period evaluated

Patient	Findings	Baseline	1 week	2 weeks	1 month	3 months	6 months	12 months
1	Visual acuity	LP	LP	LP	LP	LP	LP	LP
	IOP, mmHg	18	16	18	13	12	12	13
	Average RNFL, μm	39.9	46.2	46.6	46.5	45.8	46.0	47.1
	VF mean deviation, dB	- 18.2	- 17.9	- 19.9	- 18.5	- 18.6	- 18.7	- 18.9
2	Visual acuity	LP	LP	LP	LP	LP	LP	LP
	IOP, mmHg	12	12	08	10	12	11	10
	Average RNFL, μm	27.9	34.5	-	-	-	-	-
	VF mean deviation, dB	- 22.2	- 25.7	-	-	-	-	-

LP light perception, IOP intraocular pressure, RNFL retinal fiber layer thickness from spectral-domain optical coherence tomography, VF visual field

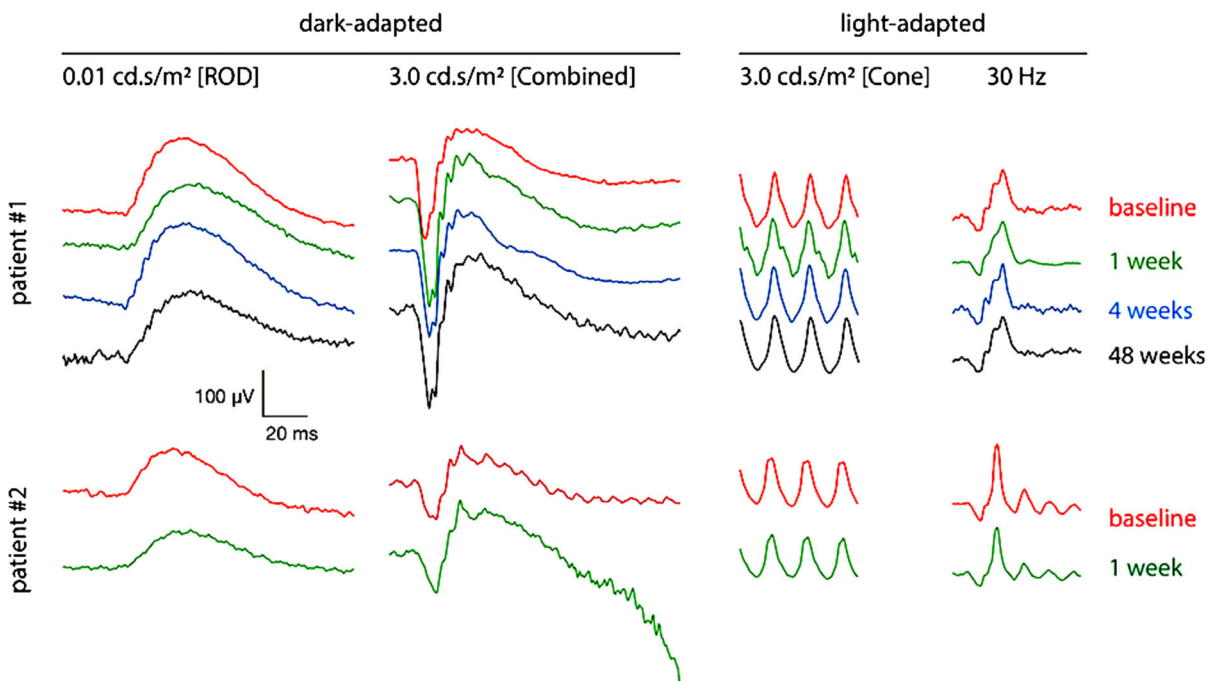


Fig. 1 ERG responses at baseline (red), and at week 1 (green), 4 (blue), and 48 (black) after injection elicited with ISCEV-recommended stimuli from the 2 subjects

ERG responses stable throughout the study period with no signs of retinal abnormalities (Figs. 1 and 3).

Retinal detachment was promptly treated with a successful pars plana vitrectomy and silicone oil tamponade, but the patient was removed from the trial, and ERG was no longer performed. At the final follow-up period (12 months), he remained with no retinal detachment and visual acuity measured as light perception.

Discussion

We described two different outcomes observed after intravitreal injections of bone marrow MSCs in human eyes, highlighting the concerns related to this procedure's safety. Although MSCs therapy has been previously associated with improvements in the retinal function, as in experimental retinal dystrophy [9] and

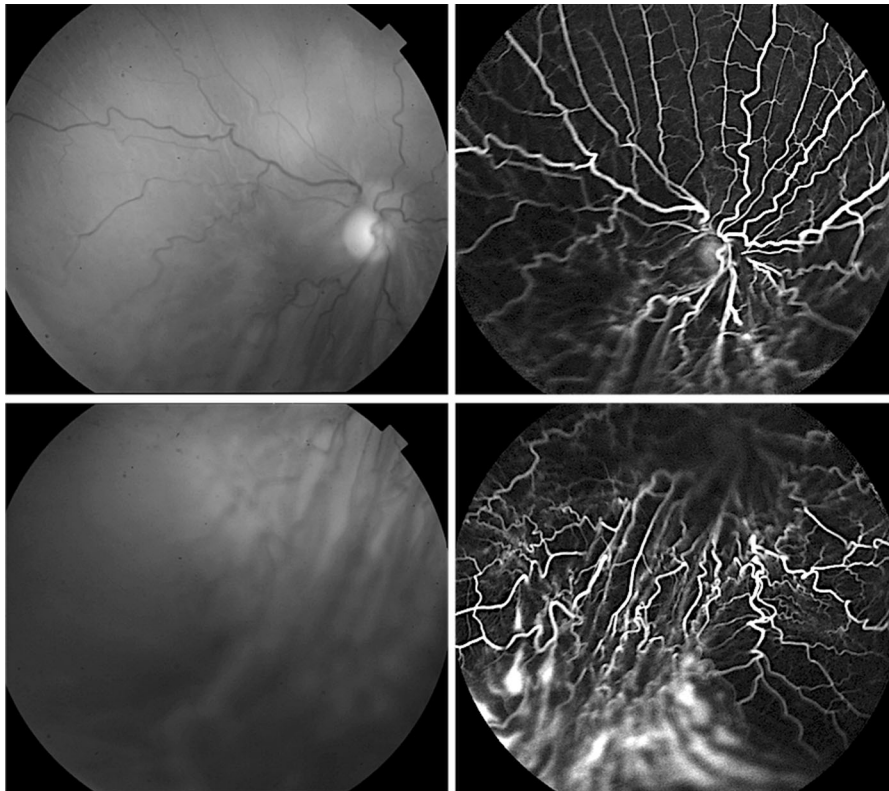


Fig. 2 Fundus images (left images) and retina fluorescein angiography (right images) of patient 2 taken after 30 days of the intravitreal injection of autologous bone marrow mesenchymal stromal cells (right eye, 1.10^6 cells). *Top images*: total retinal detachment including the macular region, with mild folds observed in the inferior retina and no hemorrhages or significant

vitreous haze. *Bottom images*: inferotemporal images showing more evident retinal folds and a yellowish diffuse subretinal exudative fluid, with fluorescein pooling in the periphery. *Note*: a moderate vitreoretinal proliferation in this region was detected during the *pars plana* vitrectomy

assessed by ERG in glaucoma [10], the safety of MSCs injections in humans was still unknown.

After bone marrow-derived MSCs treatment, ERG outcomes showed no functional improvement that could have been detected, for instance, with an increase in the photopic-negative response amplitude. Although some PhNR variation was detected during follow-up in patient 1, our study was not designed to evaluate such effects since only two advanced glaucoma cases were included. Nonetheless, ERG did not deteriorate up to 7 days after injection for both cases, indicating retinal safety of intravitreal MSCs.

The vitreous-retinal proliferative changes observed in the second case seem to be responsible for the retinal detachment. The intravitreal injection was done uneventfully in the superior temporal quadrant - with no bleeding or retinal breaks observed after the procedure - and the retinal detachment was observed

in the inferior region two weeks later when vitreous-retinal proliferative changes were also present.

A proinflammatory effect might have been the cause and has previously been reported after autologous stem cells injected into the vitreous cavity, including retinal detachment and severe proliferative vitreoretinopathy [2, 11]. In this context, it would be rational to think that pluripotent stem cells injected intravitreally may differentiate into myofibroblast-like cells [12], which could contribute to vitreous fibrosis and contraction, facilitating eventual vitreoretinal tractions. Nevertheless, this cellular differentiation could be controlled in future trials.

Of interest, since ERG responses were kept stable until the retinal detachment was observed in one case and throughout the other case's follow-up, we believe that products from MSCs are well tolerated into the vitreous cavity. They might have an eventual

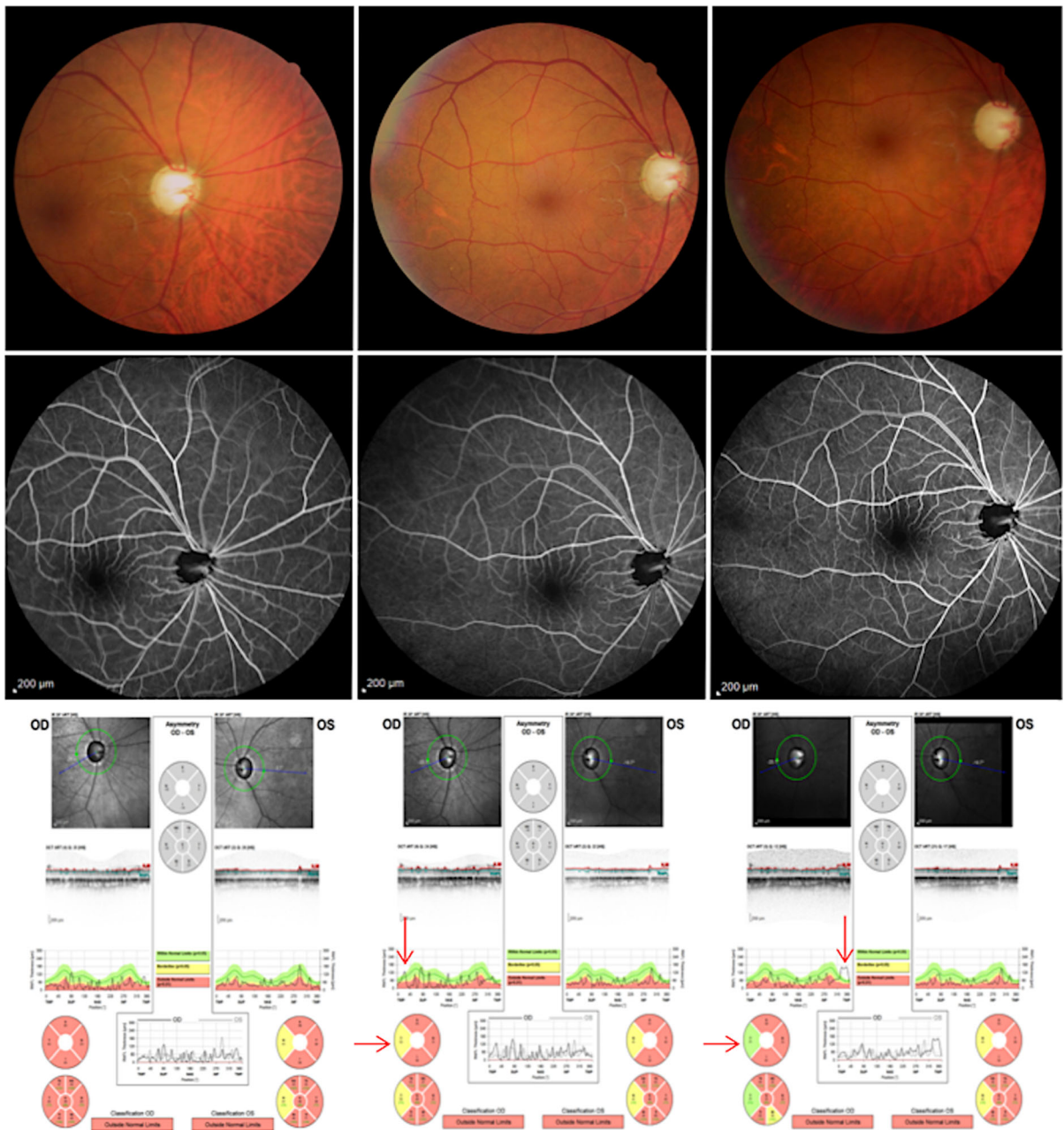


Fig. 3 Color fundus images (top), retina fluorescein angiography (middle), and optical coherence tomography results (bottom) of patient 1, before (left) and after 4 weeks (center), and 48 weeks (right) the intravitreal injection of autologous bone marrow mesenchymal stromal cells (right eye, 1.10^6 cells).

neuroprotective effect on the retina (not observed here) and caused no function damage, but the complications observed may be attributed to the

Besides the advanced cup/disk ratio, no differences were detected between the three moments of all these examinations. Note segmentation artifacts in the retinal nerve fiber layer thickness analyses of the temporal region (right eye, red arrows)

cellular proliferation and fibrosis observed in the vitreous of patient 2.

Even with the ERG not showing retinal function loss after intravitreal MSCs, the risk of severe adverse

effects and the absence of any visual improvement suggest that further studies using modified MSCs are required before considering their clinical use in ocular neurodegenerative diseases, including glaucoma.

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Availability of data and material The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Compliance with ethical standards

Statement of human rights Informed consent was obtained from all individual participants included in the study.

Consent for publication All authors have had full access to the entire review and agree with the decision to submit for publication.

Conflict of interest Authors do not have any commercial or proprietary conflicting, competing interests in the materials or products described (directly or indirectly related to) in the article. The results presented in this manuscript were not presented at any previous scientific meeting.

Ethics approval All procedures performed in this study were approved by the Ribeirão Preto Clinical Hospital Ethical Committee (process number 14151/2010) and were in accordance with Helsinki Declaration (1964) and its later amendments.

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