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GLAUCOMA

Treatment with citicoline eye drops enhances retinal function and neural conduction along the visual pathways in open angle glaucoma

Vincenzo Parisi[1] · Marco Centofanti[1,2] · Lucia Ziccardi[1] · Lucia Tanga[1] · Manuele Michelessi[2] · Gloria Roberti[1] · Gianluca Manni[2]

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Abstract
Purpose To evaluate the retinal function and the neural conduction along the visual pathways after treatment with citicoline eye drops in patients with open angle glaucoma (OAG).
Methods Fifty-six OAG patients (mean age 52.4±4.72 years, IOP <18 mmHg with beta-blocker monotherapy only) were enrolled. Of these, 47 eyes completed the study: 24 OAG eyes were treated with topical citicoline (OMK1®, Omikron Italia, 3 drops/day) (GC eyes) over a 4-month period (month 4) followed by a 2-month period of citicoline wash-out (month 6), and another 23 OAG eyes were only treated with beta-blocker monotherapy (GP eyes). In GC and GP eyes, pattern electroretinogram (PERG) and visual evoked potentials (VEP) were assessed at baseline and at months 4 and 6 in both groups.
Results At baseline, similar (ANOVA, $p>0.01$) PERG and VEP values in GC and GP eyes were observed. After treatment with topical citicoline, a significant ($p<0.01$) increase of PERG P50-N95 and VEP N75-P100 amplitudes, and a significant ($p<0.01$) shortening of VEP P100 implicit times were found. In GC eyes, the shortening of VEP P100 implicit times was correlated significantly ($p<0.01$) with the increase of PERG P50-N95 amplitudes. After a 2-month period of topical Citicoline wash-out, PERG and VEP values were similar ($p>0.01$) to baseline ones. GP eyes showed not significant changes of PERG and VEP values during the entire follow-up.
Conclusions Topical treatment with citicoline in OAG eyes induces an enhancement of the retinal bioelectrical responses (increase of PERG amplitude) with a consequent improvement of the bioelectrical activity of the visual cortex (shortening and increase of VEP implicit time and amplitude, respectively).

Keywords Glaucoma · PERG · VEP · Citicoline

Introduction

Open angle glaucoma (OAG) is a chronic disease characterized by visual field loss associated with typical optic nerve damage [1]. The reduction of the intraocular pressure (IOP) has been proven effective in reducing the conversion from ocular hypertension to OAG and in slowing the progression of glaucomatous visual dysfunction [2–4].

In OAG patients, electrophysiological recordings may provide selective information about a functional impairment occurring in the retinal preganglionic elements (abnormal flash or multifocal electroretinogram—ERG) [5, 6], in ganglion cells and their fibers (abnormal pattern ERG-PERG and reduced photopic negative response of the flash and focal ERG-PhNR) [7–14], or in the neural conduction along the visual pathways (abnormal visual evoked potentials—VEP) [12, 15, 16].

The possibility of influencing the progression of visual dysfunction has constituted a constant effort for years. Toward this end, an improvement of PERG responses may be obtained

✉ Lucia Ziccardi
luxzic@hotmail.com

[1] "G.B. Bietti" Foundation for Study and Research in Ophthalmology - IRCCS, Via Livenza 3, 000198 Rome, Italy

[2] Department of Clinical Sciences and Translational Medicine, "Tor Vergata" University of Rome, Via Montpellier 1, 00133 Rome, Italy

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after lowering the IOP with beta-blockers or acetazolamide [17–22] and following treatment with nicergoline [23], or with coenzyme Q10 in conjunction with vitamin E [24].

Since 1996, we posed our attention on the possibility of obtaining an improvement of glaucomatous visual dysfunction using a pharmacological approach similar to that used in different brain disorders due to vascular, traumatic or degenerative processes (see Secades and Lorenzo for a review [25]), and we found that after intramuscular or oral treatment with citicoline, OAG patients had an improvement of ganglion cell function and of neural conduction along the visual pathways [26–28]. Thus, Chang and Goldberg [29] have suggested that citicoline may have a neuroenhancing effect that could explain the amelioration of the glaucomatous perimetric condition [30] and the reduction of the progression of visual field defects as recently reported in glaucomatous eyes (by using 500 mg of citicoline in oral solution) [31]. Beneficial effects of citicoline in other conditions of visual dysfunction, such as amblyopia [32, 33] and non-arteritic ischemic optic neuropathy [34], have also been reported.

Citicoline is also currently available for topical treatment. It is documented that in an animal experimental model using high performance liquid chromatography (HPLC) assessment, citicoline reaches the vitreous (using as vehicles high molecular weight hyaluronic acid and benzalkonium chloride as penetration enhancers) [35], and therefore, there is a real possibility that this substance may act directly on those ocular elements close to the posterior chamber (ganglion cells and their fibers) that are morpho-functionally affected in glaucomatous disease.

Thus, the aim of our study was to evaluate, through the use of electrophysiological methods (PERG and VEP recordings), whether topical treatment with citicoline could have an effect on the retinal function and on neural conduction along the visual pathways in patients with OAG. In addition, by inducing a period of wash-out, our study also intended establishing whether or not the potential effects were dependent on the topical treatment with citicoline.

Materials and methods

Patients

Fifty-six eyes from 56 patients (mean age 52.4±4.72 years, range: 40–60 years) affected by OAG were recruited. OAG patients were selected from a larger population of 238 OAG patients on the basis of the following inclusion criteria:

- Intraocular pressure (IOP) >23 and <28 mmHg (average of the two highest readings of the daily curve, from 8:00 a.m. to 6:00 p.m., six independent readings, one every 2 h) without medical treatment;
- Humphrey Field Analyzer (HFA) 24/2 Mean Deviation (MD) >- 10 dB; pattern standard deviation (PSD) <10 dB; fixation losses, false positive rate, and false negative rate each less than 20 %;
- Best corrected visual acuity (BCVA) ranging from 0.0 to 0.1 logMAR;
- One or more papillary signs on conventional color stereo slides: the presence of a localized loss of neuroretinal rim (notch), thinning of the neuroretinal rim, generalized loss of optic rim tissue, optic disc excavation, vertical or horizontal cup/disc ratio greater than 0.5, cup-disc asymmetry between the two eyes greater than 0.2, peripapillary splinter hemorrhages;
- Refractive error (when present) between −3.00 and +3.00 spherical equivalent;
- No previous history or presence of any disease involving cornea, lens, macula or retina;
- No previous history or presence of diabetes, optic neuritis, or any disease involving the visual pathways;
- Pupil diameter ≥3 mm without mydriatic or miotic drugs;
- Central corneal thickness, assessed by ultrasonic pachymetry performed using AL 2000 Bio & Pachymeter (Tomey Corporation, Japan), within 500 and 600 μm.

Since it is known that PERG responses can be modified by a pharmacological reduction of IOP [17–22], we only enrolled OAG patients with IOP values less than 18 mmHg on beta-blocker monotherapy which was maintained during the 8 months preceding the first electrophysiological evaluation and during the whole study. IOP was assessed as the average of the two highest readings of the daily curve (see above).

When both eyes in an OAG patient met the above-mentioned inclusion criteria, we included only one eye from each patient: the eye with the greater retinal ganglion cells (RGCs) dysfunction identified by the lower values of the PERG P50-N95 amplitude (see below).

Twenty-five eyes of 25 normal age-similar subjects (range: 42–60 years, mean age 51.4±4.09 years) provided control data.

Study design

This study has been designed as a randomized, prospective, and masked study. The research followed the tenets of the Declaration of Helsinki and the study was approved by the local ethics committee (Azienda Sanitaria Locale Roma A). Upon recruitment, each patient was aware that he/she was being enrolled in a study to test a new topical drug and provided an informed consent.

1) Baseline. All enrolled patients were randomly divided into two age-similar groups each made up of 28 patients: the GP group (23 enrolled eyes, see below; mean age 52.7 ±4.78, age range: 42–60 years) and the GC Group (24

enrolled eyes, see below; mean age 52.1±4.66, age range: 42–60 years). The random separation of citicoline-treated and citicoline-not-treated patients (enrolled by MC, LT, GR, MM, and GM) was performed by an electronically generated randomization system on the basis of age, gender, IOP, and HFA MD. The key was opened to all the investigators at the end of the follow-up period. Table 1 reports the clinical characteristics of Control, GC, and GP eyes at baseline condition.

2) Months 0–4: 4-month period of treatment with topical citicoline. Throughout a 4-month period, GP eyes were treated exclusively with beta-blocker monotherapy, while GC eyes, in addition to the topical treatment with beta-blockers, received topical treatment with OMK1® (citicoline sodium salt: 0.2 g, hyaluronic acid: 0.02 g, benzalkonium chloride 0.001 g, water for injection up to 10 ml, OMK1®, Omikron Italia, Italy, 3 drops /day). During this period, four eyes belonging to the GC group were excluded for different reasons: two due to lack of compliance, one due to an increase of IOP (23 mmHg), and one due to the patient's change of mind about participating in the study; therefore, 24 GC eyes completed the study. Five eyes belonging to the GP group were excluded: two due to lack of compliance and three due to an increase of IOP (>21 and <24 mmHg); therefore, 23 GP eyes completed the study.
3) Months 5–6: 2-month period of topical citicoline wash-out. After a 4-month period of topical citicoline treatment, GC eyes continued exclusively the topical treatment of beta-blockers, performing a 2-month period of topical citicoline wash-out. During the same period, GP eyes were treated exclusively with beta-blocker monotherapy.

Following a criterion previously used in other published works [24, 34], in order to evaluate the PERG and VEP responses (see below) independently from the clinical conditions of the tested subjects, all electrophysiological examinations were performed at baseline condition and after 4 and 6 months of follow-up in the presence of two operators (VP, LZ) who were masked during the group treatment for each patient.

Compliance to eye drops administration was assessed through a questionnaire, which was distributed by the study personnel during each visit. As expected in a clinical study, reported adherence to treatment was high, and all patients rated their compliance as "good to very good" (regular use of the eye drops in at least 80 % of the trial period).

Electrophysiological examinations

In Controls, GC, and GP eyes, the electrophysiological examination was performed at baseline and in GC and GP eyes after 4 and 6 months of follow-up.

In agreement with previously published studies [11, 12, 24, 26–28, 36, 37], simultaneous PERG and VEP recordings were performed using the methods described below.

Subjects were seated in a semi-dark, acoustically isolated room in front of the display surrounded by a uniform field of luminance of 5 cd/m^2. Prior to the experiment, each subject was adapted to the ambient room light for 10 min, with a pupil diameter, measured by a ruler, of approximately 5 mm. Mydriatic or miotic drugs were never used. Stimulation was monocular after occlusion of the fellow eye. Visual stimuli were checkerboard patterns (contrast 80 %, mean luminance 110 cd/m^2) generated on a television monitor and reversed in contrast at the rate of two reversals per second. At a viewing distance of 114 cm, the check edges subtended 15 min (15′) of visual angle, while the monitor screen subtended 18°. PERG and VEP recordings were performed with full correction of refraction at the viewing distance. The visual stimuli is different form the one suggested by the ISCEV standards (i.e., 0.8° for PERG and 60 and 15 min of visual angle for VEP) [38, 39]. We adopted the same visual stimuli (15 min of visual arc, contrast of 80 % and a rate of two reversals per second), which allowed us to detect the highest sensitivity and specificity of PERG and VEP in detecting retinal and post-retinal dysfunction in glaucomatous eyes (see for a review Parisi et al. [37]). A small red fixation target, subtending a visual angle of approximately 0.5° (estimated after taking into account spectacle-corrected individual refractive errors) was placed at the centre of the pattern stimulus. At every PERG and VEP examination, each patient positively reported that he/she could clearly perceive the fixation target.

PERG recordings

The bioelectrical signal was recorded by a small Ag/AgCl skin electrode placed over the lower eyelid. PERGs were bipolarly derived between the stimulated (active electrode) and the patched (reference electrode) eye using a previously described method [40]. As the recording protocol was extensive, the use of skin electrodes with interocular recording represented a good compromise between the signal-to-noise ratio and signal stability. A discussion on PERG using skin electrodes and its relationship to the responses obtained by corneal electrodes can be found elsewhere [41, 42]. The ground electrode was in Fpz [43]. Interelectrode resistance was lower than 3000Ω. The signal was amplified (gain 5,0000), filtered (band pass 1-30 Hz), and averaged with an automatic rejection of artifacts (200 events free from artifacts were averaged for every trial) by BM600 (Biomedica Mangoni, Pisa, Italy). Analysis time was 250 ms.

The transient PERG response is characterized by a number of waves with three subsequent peaks of negative, positive, and negative polarity, respectively. In visually normal subjects, these peaks have the following implicit times: 35, 50,

Table 1 Clinical characteristics at baseline condition observed in Control subjects (C, *N*=25), in OAG patients treated with beta-blockers plus topical citicoline (GC eyes, *N*=24), and in OAG patients treated exclusively with beta-blockers (GP group, *N*=23 eyes). Age (years), visual acuity (VA, LogMAR), intraocular pressure at the time of the first diagnosis of ocular hypertension (IOP-F. mmHg), Humphrey 24-2 visual field Mean Deviation (MD, dB) and Pattern Standard Deviation (PSD, dB), time elapsed from the diagnosis of OAG (TE, months). ANOVA, Statistical evaluation by a one-way analysis of variance; *SD* 1 standard deviation

		Mean±SD	ANOVA vs. Controls	ANOVA vs. GP
Age	C	51.4±4.09		
	GC	52.1±4.66	f(1,48): 0.31; p=0.578	f(1,46): 0.19; p=0.665
	GP	52.7±4.78	f(1,47): 1.03; p=0.307	
VA	C	0.004±0.018		
	GC	0.009±0.024	f(1,48): 0.68; p=0.412	f(1,46): 0.02; p=0.896
	GP	0.008±0.028	f(1,47): 0.35; p=0.556	
IOP-F	C	13.8±1.98		
	GC	26.8±1.58	f(1,48): 641.9; p<0.0001	f(1,46): 0.36; p=0.550
	GP	27.1±1.83	f(1,47): 581.0; p<0.0001	
MD	C	−0.56±0.82		
	GC	−4.78±2.85	f(1,48): 50.50; p<0.0001	f(1,46): 0.01; p=0.951
	GP	−4.83±2.74	f(1,47): 55.42; p<0.0001	
PSD	C	1.17±0.28		
	GC	4.93±3.33	f(1,48): 31.67; p<0.0001	f(1,46): 0.01; p=0.945
	GP	4.86±3.58	f(1,47): 26.43; p<0.0001	
TE	C	–		
	GC	21.8±3.42		f(1,46): 1.14; p=291
	GP	22.9±3.64		

and 95 ms (N35, P50, N95). PERG P50-N95 peak-to-peak amplitudes were measured for each of the averaged waves directly on the displayed records by means of a pair of cursors.

VEP recordings

Cup shaped electrodes of Ag/AgCl were fixed with collodion in the following positions: active electrode in Oz [43], reference electrode in Fpz [43], ground on the left arm. Interelectrode resistance was kept below 3000Ω. The bioelectric signal was amplified (gain 2,0000), filtered (band pass 1–100 Hz) and averaged (200 events free from artifacts were averaged for every trial) by BM6000 (Biomedica Mangoni, Pisa, Italy). Analysis time was 250 ms. The transient VEP response is characterized by a number of waves with three subsequent peaks of negative, positive, and negative polarity, respectively. In visually normal subjects, these peaks have the following implicit times: 75, 100, and 145 ms (N75, P100, N145).

VEP P100 implicit times and N75-P100 peak-to-peak amplitudes were measured for each of the averaged waves directly on the displayed records by means of a pair of cursors.

During a recording session, simultaneous PERG and VEP were recorded at least twice (between two to six times) and the resulting waveforms were superimposed to check the repeatability of results. On the basis of previous studies [24, 37], we know that intra-individual variability (evaluated by test-retest) is approximately±2 ms for VEP P100 implicit times and approximately±0.18 μV for PERG P50-N95 and VEP N75-P100 amplitudes. During the recording session we considered "superimposable", and therefore, repeatable, two successive waveforms, with a difference in ms (for VEP P100 implicit times) and in μV (for PERG P50-N95 and VEP N75-P100 amplitudes) that was less than the above reported values of intra-individual variability. At times, the first two recordings were sufficient to obtain repeatable waveforms, while other times, further recordings were required (albeit never more than six in the cohort of patients). For statistical analyses (see below), we considered PERG and VEP values measured in the recording with the lowest PERG P50-N95 amplitude.

In each patient, the signal-to-noise ratio (SNR) of PERG and VEP responses was assessed by measuring a "noise" response while the subject fixated an unmodulated field of the same mean luminance as the stimulus. At least two "noise" records of 200 events each were obtained, and the resulting grand average was considered for measurement. The peak-to-peak amplitude of this final waveform (i.e., the average of at least two replications) was measured in a temporal window corresponding to the same amplitude at which the response component of interest (i.e., VEP N75-P100, PERG P50-N95) was expected to peak. For this component, SNRs were determined by dividing the peak amplitude of the noise component in the corresponding temporal window. We observed an

electroretinographic noise <0.1 μV (mean 0.079 μV, range 0.066 to 0.089 μV, resulting from the grand average of 400–1200 events), and an evoked potential noise < 0.15 μV (mean 0.097 μV, range 0.079 to 0.113 μV, resulting from the grand average of 400–1200 events) in all tested subjects. Moreover, we accepted VEP and PERG signals with a signal-to-noise ratio > 2 for all subjects and patients.

Statistics

Sample size estimates were obtained from pilot evaluations performed in 20 eyes from 20 OAG eyes and 20 eyes from 20 control subjects, other than those included in the current study (unpublished data). Inter-individual variability, expressed as standard deviation (SD) data, was estimated for PERG P50-N95 amplitude and VEP P100 implicit time measurements. It was found that for PERG P50-N95 amplitude, SD values were significantly higher for patients (mean, 0.56 μV; SD, 0.19 μV, about 34 % of the mean) when compared to controls (mean, 1.29 μV; SD, 0.19 μV, about 15 % of the mean). Assuming the above, it was also established that, among SD subjects (35 % since they were all OAG patients) in the current study, sample sizes of control subjects and patients belonging to the OAG group provided a power of 90 %, at an alpha of 0.05, detecting a difference between groups of 55 % or greater in PERG P50-N95 amplitude. They were also expected to be electrophysiologically significant when comparing results of treated OAG eyes observed at baseline conditions versus those observed at 4 and 6 months.

Test-retest data (obtained in the group of OAG patients evaluated in this study) of PERG and VEP results were expressed as the mean difference between two recordings obtained in separate sessions±SD of this difference. A 95 % confidence limit (CL, mean±2 SD) of test-retest variability in OAG patients was established assuming a normal distribution.

The differences of PERG and VEP response values between groups (GC, GP, and Control eyes) were evaluated by a one-way analysis of variance (ANOVA). Changes in the PERG and VEP responses that were observed in GC eyes and GP eyes when compared to baseline were evaluated by ANOVA for repeated measures. After the different treatments, the differences observed in individual OAG eyes with respect to the baseline values, were calculated performing a logarithmic transformation to better approximate a normal distribution. Pearson's correlation was used to correlate the changes during the follow-up of all electrophysiological parameters (PERG and VEP values) with baseline PERG and VEP values, age, time elapsed from the diagnosis OAG, IOP at the time of the first diagnosis of ocular hypertension, IOP at the time of electrophysiological examination, MD, and PSD.

In order to compensate for multiple comparisons, a conservative p-value less than 0.01 was considered statistically significant in all the analyses.

Results

Figure 1 shows examples of simultaneous PERG and VEP recordings and relative HFA performed in one OAG patient that was treated with beta-blocker monotherapy and additional treatment with topical Citicoline for 4 months, followed by a 2-month period of topical citicoline wash-out.

Table 2 outlines the mean data of PERG and VEP parameters observed in Controls, GC, and GP groups at baseline and the number of normal or abnormal GC and GP eyes.

Individual PERG and VEP changes observed in GC and GP eyes at 4 and 6 months compared to baseline are shown in Figs. 2a and 3a, respectively. Table 3 lists the number of individual changes expressed in absolute values and percentages with respect to the total number of eyes belonging to GC and GP groups at months 4 and 6 of follow-up.

The mean of individual PERG and VEP changes observed in GC and GP groups at 4 and 6 months compared to baseline and the relative statistical analyses between GC and GP groups are presented in Table 4.

The mean data of PERG and VEP parameters observed in GC and GP groups at baseline and after 4 and 6 months are shown in Figs. 2b and 3b, respectively. The relative statistical analyses are presented in Table 5.

Figure 4a and b present the correlations found in GC eyes between PERG P50-N95 amplitude differences (4 months minus baseline) and baseline values, and the differences (4 months minus baseline) of VEP P100 implicit times, respectively.

Months 0–4: 4 month-period of treatment with topical citicoline

When considering the individual changes concerning the 95 % CL after 4 months of topical citicoline treatment, a large percentage of GC eyes showed an increase of PERG P50-N95 amplitudes (79 %) and a shortening of VEP implicit times (79 %), while 50 % of GC eyes showed unmodified or improved VEP N75-P100 amplitudes. In the GP group, the majority (more than 86 %) of eyes presented electrophysiological parameters substantially unmodified with respect to those observed at baseline (see Table 3, Figs. 2a and 3a).

In GC eyes, the better improvement of PERG P50-N95 amplitudes was significantly ($p<0.01$) correlated with the larger impairment at baseline (see Fig. 4a). The changes in VEP P100 implicit times and VEP N75-P100 amplitude values were independent ($p>0.01$) from the baseline condition.

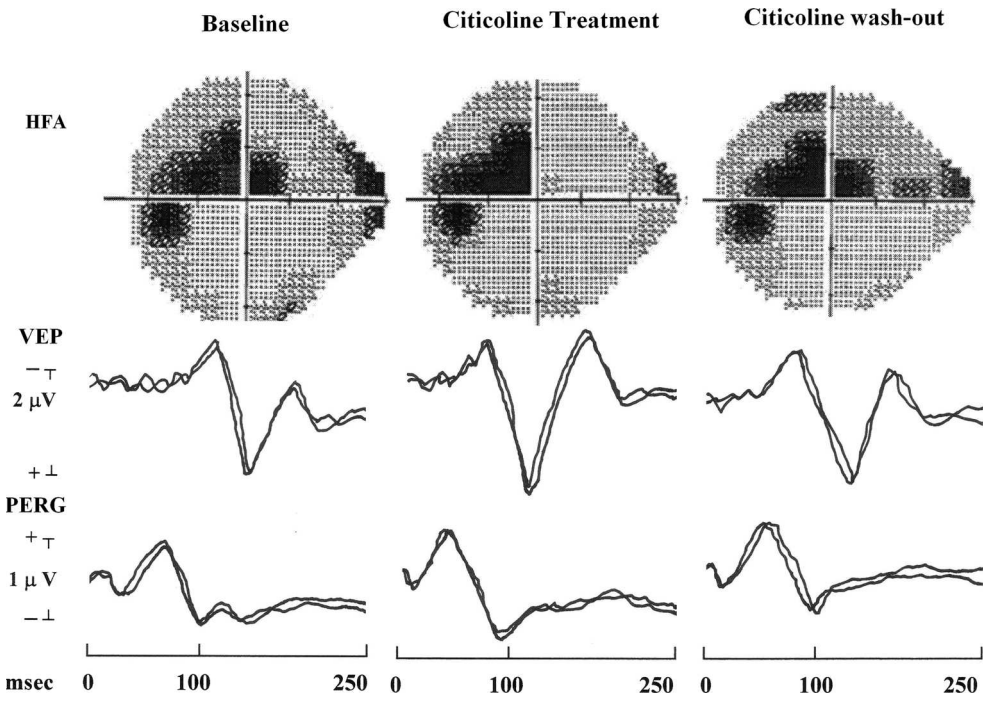

Fig. 1 Examples of simultaneous VEP and PERG recordings and Humphrey Field Analyzer (HFA) performed in one OAG eye treated with beta-blockers plus citicoline at baseline (baseline), at the end of treatment with beta-blockers plus citicoline (month 4, citicoline treatment) and at the end of the citicoline wash-out period and thus only treated with beta-blockers (month 6, citicoline wash-out). In comparison with the baseline condition, after treatment with citicoline, an increase in amplitudes in PERG recordings, a shortening of implicit times and an increase of amplitudes in VEP recordings, together with changes of the visual field defects can be observed. At the end of the citicoline wash-out period, the electrophysiological and HFA findings were similar to those at baseline

The improvement of VEP implicit time was significantly ($p<0.01$) correlated with the changes of PERG P50-N95 amplitudes (see Fig. 4b).

Non-significant ($p>0.01$) correlations were observed between the differences of all electrophysiological parameters (PERG and VEP values) and age, the time elapsed from the diagnosis of OAG, IOP at the time of the first diagnosis of ocular hypertension, IOP at the time of electrophysiological examination, MD, and PSD.

On average, in the GC group, the mean of individual changes in PERG P50-N95 and VEP N75-P100 amplitudes, and VEP P100 implicit times were significantly ($p<0.01$) different with respect of those observed in GP group (see Table 4). In the GC group, the mean values of PERG P50-N95 and VEP N75-P100 amplitudes and VEP P100 implicit times were significantly ($p<0.01$) increased and reduced respectively when compared with those observed at baseline. In the GP group non-significant ($p>0.01$)

Table 2 Mean values of PERG P50-N95 amplitudes and VEP P100 implicit times and N75-P100 amplitudes detected at baseline in Control subjects (C, $N=25$ eyes), in OAG patients treated with beta-blockers plus topical citicoline (GC group, $N=24$ eyes), and in OAG eyes treated exclusively with beta-blockers (GP group, N=23 eyes). ANOVA, Statistical evaluation by a one-way analysis of variance versus Controls

	Group	Mean±SD	ANOVA vs. Controls	Nr	Ab
PERG P50-N95 A (microV)	C	2.09±0.24			
	GC	1.15±0.33	f(1,48): 130.82; $p<0.001$	0	24
	GP	1.08±0.33	f(1,47): 148.78; $p<0.001$	0	23
VEP P100 IT (ms)	C	107.8±3.36			
	GC	128.5±7.45	f(1,48): 159.35; $p<0.001$	0	24
	GP	126.8±7.14	f(1,47): 142.86; $p<0.001$	0	23
VEP N75-P100 A (microV)	C	16.3±2.36			
	GC	6.83±2.50	f(1,48): 186.04; $p<0.001$	0	24
	GP	6.33±1.74	f(1,47): 273.49; $p<0.001$	1	22

SD 1 standard deviation. *IT* Implicit Time, *A* Amplitude. *Nr* number of eyes inside the normal limits. *Ab* number of eyes outside the normal limits. Normal limits were obtained from control subjects by calculating mean values + 2 standard deviations for VEP P100 implicit and mean values –2 standard deviations for PERG P50-N95 and VEP N75-P100 amplitudes

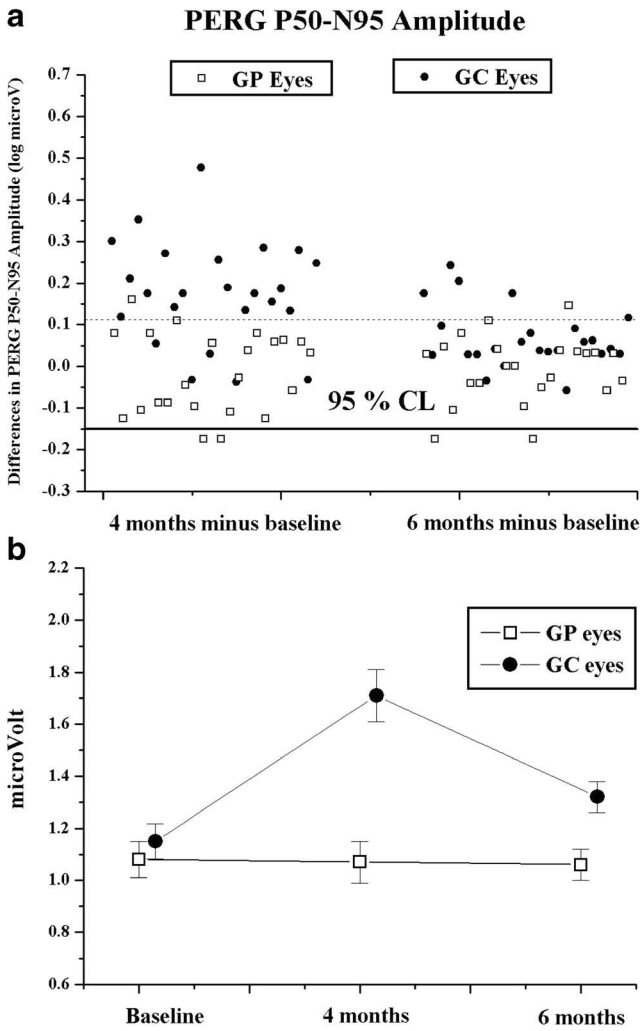

Fig. 2 PERG P50-N95 Amplitude individual changes (**a**) and graphic representation of mean values (**b**) observed in OAG patients treated with beta-blocker monotherapy plus citicoline (GC group, *N*=24 eyes) and in OAG patients treated exclusively with beta-blocker monotherapy (GP group, *N*=23 eyes). The percentage of unmodified eyes (within the 95 % confidence test-retest limit, CL), eyes with improvement (values over the 95 % confidence test-retest limit, *dashed line*) and eyes with worsening (values under the 95 % confidence test-retest limit for amplitudes, *solid line*) is reported on Table 3. The statistical changes for PERG amplitude values are reported in Table 5. *Vertical lines,* one error standard

changes in mean values of PERG and VEP parameters were found (see Table 5).

On the basis of ancillary findings, it was interesting to observe that during the same period of study, several GC eyes (17 out of 24) showed changes in the visual field parameters: MD values with an increase greater than 1.0 dB with respect to baseline and PSD values with a reduction greater than 1.0 dB when compared to baseline ones. As a consequence, the individual increment in MD induces a positive mean progressive rate (0.56 dB). In all GP eyes (23 out of 23), MD and PSD values showed changes lower than 1.0 dB with respect to baseline, with consequent mean progressive rate of −0.24 dB. In Fig. 4c and d are presented the correlations observed in GC eyes between the differences (4 months minus baseline) in PERG P50 amplitude and in VEP P100 implicit times and the corresponding differences in MD values. The MD changes were significantly ($p<0.01$) correlated with the differences in PERG P50-N95 amplitude and VEP P100 implicit time. A qualitative example of this finding is reported in Fig. 1.

However, since the power of our study was defined on the basis of the main electrophysiological parameter (i.e., PERG P50-N95 amplitude) and considering the HFA MD and PSD inter-individual variability at baseline, we believe that it is not adequate to provide a statistical evaluation about the HFA MD and PSD changes. In fact, to obtain valid differences in the visual field parameters, a larger cohort of OAG patients would be required.

Months 5–6: 2-month period of topical citicoline wash-out

In the GC eyes cohort, a large percentage (ranging from 79 to 87 %) of the individual values of PERG and VEP parameters were within the 95 % CL and, therefore, unmodified with respect to baseline. In GP group, a percentage of more than 86 % of eyes showed values of PERG and VEP parameters substantially unmodified with respect to baseline ones (see Table 3, Figs. 2a and 3a).

On average, in the GC group, the mean of individual changes in VEP N75-P100 amplitudes were similar ($p>0.01$) to those observed in GP group, while the mean of individual changes in VEP P100 implicit time and PERG P50-N95 amplitudes were still significantly ($p<0.01$) different with respect thos of the GP group (see Table 4). In the GC and GP groups, the mean values of all electrophysiological parameters were not significantly ($p>0.01$) different with respect to those detected at baseline (see Table 5).

As outlined in the example of Fig. 1, in the same follow-up period, several GC eyes (22 out of 24) showed a change in the HFA MD and PSD individual values that was less than 1.0 dB when compared to baseline values. Therefore, with respect to the baseline, the mean progressive rate was still positive (0.18 dB), but reduced with respect to those at 4 months of follow-up. All GP eyes (23 out of 23) showed MD and PSD values with changes lower than 1.0 dB with respect to baseline (mean progressive rate of −0.34 dB).

Throughout the entire period of treatment with topical citicoline, and after the 2-month period of topical Citicoline wash-out, no ocular adverse side effects or significant changes ($p>0.01$) in IOP or in visual acuity, were detected in any of the eyes that concluded the study. In addition, at 4 and 6 months of follow-up, in GC eyes (including the only one eye excluded for the increase in IOP at 23 mmHg) and in GP eyes (including the three eyes with an increase in IOP>21 and <24 mmHg), non-significant ($p>0.01$) differences in IOP with respect to

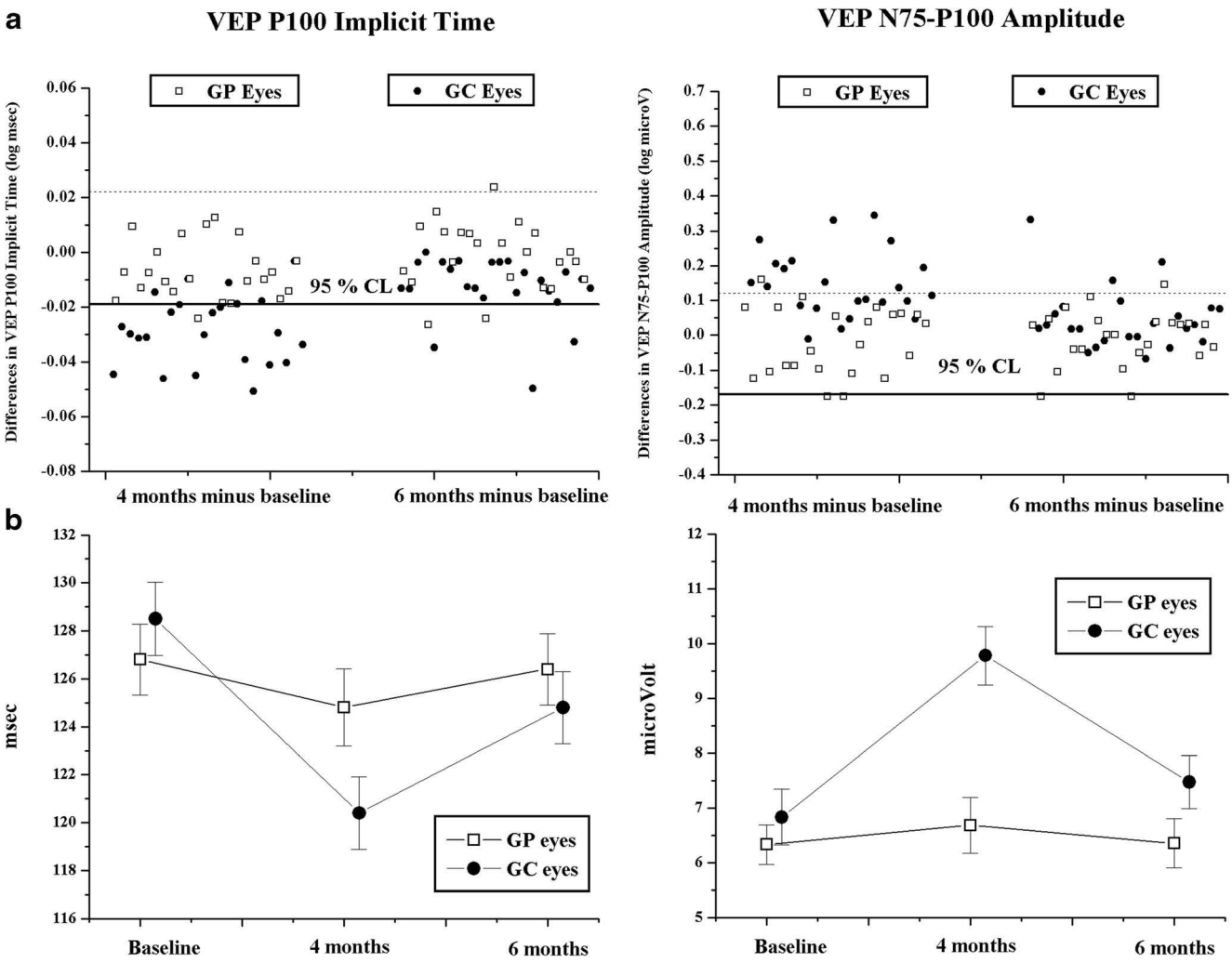

Fig. 3 VEP parameters (P100 Implicit Time and N75-P100 Amplitude) of individual changes (**a**) and graphic representation of mean values (**b**) observed in OAG patients treated with beta-blocker monotherapy plus citicoline (GC group, $N=24$ eyes) and in OAG patients treated exclusively with beta-blocker monotherapy (GP group, $N=23$ eyes). The percentage of unmodified eyes (within the 95 % confidence test-retest limit, CL), eyes with improvement (values under the 95 % confidence test-retest limit for implicit times, *solid line,* and values over the 95 % confidence test-retest limit for amplitudes, *dashed line*), and eyes with worsening (values over the 95 % confidence test-retest limit for implicit times, *dashed line*, and values under the 95 % confidence test-retest limit for amplitudes, *solid line*) is reported on Table 3. The statistical changes for the VEP values are reported in Table 5. *Vertical lines,* one error standard

baseline were found. Non-significant ($p>0.01$) differences in IOP between GC and GP eyes were found.

Discussion

The aim of our study was to evaluate whether the treatment with topical citicoline could have any effect on the retinal function and the neural conduction along the visual pathways in open angle glaucoma patients.

Retinal function (PERG data)

Glaucomatous retinal dysfunction can be assessed by PERG recordings, and the abnormal PERG responses observed in OAG patients can be ascribed to a dysfunction of the innermost retinal layers [7–12] on the basis of documented histological studies [44–46] and objective methods of in vivo morphological evaluation of retinal fibers [47, 48] showing a loss of RGCs and relative fibers. Nevertheless, a functional impairment of preganglionic elements has also been previously suggested [5, 6, 49–52]. While RCGs do not contribute to the conventional flash ERG, under specific recording conditions it is possible to obtain signals consistent with RGC activity, a method known as "photopic negative response" or PhNR recording [13, 14, 53]. In the present study, we chose to analyze only the PERG P50-N95 amplitude, because our cohort had stable fixation and absence of optical media opacity and since longitudinal studies for the clinical applications in

Table 3 Changes of electrophysiological parameters (PERG P50-N95 amplitudes, VEP P100 implicit times and VEP N75-P100 amplitudes) after 4 and 6 months of treatment with respect to the baseline condition observed in OAG patients treated with beta-blockers plus topical citicoline (GC group, *N*=24 eyes) and in OAG eyes treated exclusively with beta-blockers (GP group, *N*=23 eyes). Unmodified=within the 95 % confidence test-retest limit; improvement=values of increase in amplitudes (A) and shortening in implicit times (IT) that exceeded the 95 % confidence test-retest limit; worsening=values of reduction in amplitudes (A) and increase in implicit times (IT) that exceeded the 95 % confidence test-retest limit. *N* number of eyes

	GP eyes (23 eyes)						GC eyes (24 eyes)					
	Unmodified		Improvement		Worsening		Unmodified		Improvement		Worsening	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Difference 4 months minus baseline												
PERG P50-N95 A	20	86.95	1	4.36	2	8.69	5	20.83	19	79.17	0	0
VEP P100 IT	22	95.64	1	4.36	0	0	5	20.83	19	79.17	0	0
VEP N75-P100 A	20	86.95	1	4.36	2	8.69	12	50	12	50	0	0
Difference 6 months minus baseline												
PERG P50-N95 A	20	86.95	1	4.36	2	8.69	19	79.17	5	20.83	0	0
VEP P100 IT	20	86.95	2	8.69	1	4.36	21	87.50	3	12.50	0	0
VEP N75-P100 A	20	86.95	1	4.36	2	8.69	21	87.50	3	12.50	0	0

glaucoma are reported only for this technique [54, 55], whereas for PhNR these are not available yet [56].

In this study, we detected that the group of OAG patients treated over a 4-month period with topical citicoline showed an improvement of retinal function as suggested by the increased PERG amplitudes. A similar enhancement has been previously observed by using both oral and intramuscular treatments with citicoline [26–28]. Our results are in agreement with those presented in a brief report whereby PERG amplitude changes were detected in a restricted and different cohort of OAG patients after only 2 months of topical citicoline treatment [35].

Table 4 Mean values of the individual differences (4 months minus baseline and 6 months minus baseline) in PERG P50-N95 amplitudes, in VEP P100 implicit times, and VEP N75-P100 amplitudes observed in OAG patients treated with beta-blockers plus topical citicoline (GC group, *N*=24 eyes) and in OAG eyes treated exclusively with beta-blockers (GP group, *N*=23 eyes)

	4 months baseline		6 months baseline	
	Mean	SD	Mean	SD
Difference in PERG P50-N95 amplitude (Log microV)				
GC eyes	0.1769	0.125	0.0668	0.072
GP eyes	−0.0178	0.098	−0.0084	0.080
ANOVA GC vs. GP f(1,46)	35.1; $p<0.001$		11.4; $p=0.0015$	
Difference in VEP P100 implicit time (Log ms)				
GC eyes	−0.0283	0.013	−0.0129	0.011
GP eyes	−0.0070	0.010	−0.0013	0.012
ANOVA GC vs. GP f(1,46)	38.9; $p<0.001$		11.1; $p=0.0017$	
Difference in VEP N75-P100 amplitude (Log microV)				
GC eyes	0.1675	0.138	0.0450	0.088
GP eyes	−0.0178	0.098	−0.0084	0.080
ANOVA GC vs. GP f(1,46)	27.8; $p<0.001$		4.71; $p=0.0352$	

ANOVA, Statistical evaluation by a one-way analysis of variance between GC and GP eyes. *SD* 1 mean standard deviation

Citicoline is an endogenous substance that acts as an intermediary in the synthesis of phosphatidylcholine (a major phospholipid in the neuronal membrane) through the activation of the biosynthesis of structural phospholipids in neuronal membranes [57–60]. Citicoline increases the metabolism of cerebral structures, inhibits phospholipid degradation and induces an increase in the levels of different neurotransmitters and neuromodulators, including noradrenaline in the central nervous system [59, 60]. In addition, a mechanism of dopamine-like action has also been supposed [59]. Regarding the direct effect of citicoline on the RGCs, the literature reports two interesting works where in an experimental model of cultured retina it was possible to reduce the RGC degeneration by applying citicoline [61, 62]. Recently, on the same topic, Matteucci et al. [63] reported remarkable data on the administration of citicoline in retinal cultures treated with glutamate or high glucose (a model of neurodegeneration) and consequent decreased pro-apoptotic effects and a reduced synaptic loss.

Thus, the effect of topical citicoline on the RGC function that we observed could be ascribed to different/concomitant factors: a direct effect on the RGC structure [61–63] and/or to a neuromodulator effect on the retinal neurotransmitters (see Secades and Lorenzo for a review) [25].

According to the aim of our study, retinal morphological examination (i.e., by optical coherence tomography, OCT) was not performed and therefore we were not able to show whether there were other effects on the retinal fiber structures (i.e., a change of the retinal nerve fiber layer thickness).

Table 5 Mean values of PERG P50-N95 amplitudes, VEP P100 implicit times, and VEP N75-P100 amplitudes detected at baseline condition and after 4 and 6 months in OAG patients treated with beta-blockers plus topical citicoline (GC group, *N*=24 eyes) and in OAG eyes treated exclusively with beta-blockers (GP group, *N*=23 eyes)

					ANOVA vs. GP		ANOVA vs. baseline	
Group	*N*	Time	Mean	SD	*f*	*p*	*f*	*p*
PERG P50-N95 amplitude (microV)								
GP	23	Baseline	1.08	0.331				
GP	23	4 months	1.07	0.388			(1,45) = 0.033	0.855
GP	23	6 months	1.06	0.306			(1,45) = 0.090	0.765
GC	24	Baseline	1.15	0.334	(1,46)=0.511	0.478		
GC	24	4 months	1.71	0.490			(1,47) = 21.0	<0.001
GC	24	6 months	1.32	0.313			(1,47) = 3.03	0.088
VEP P100 implicit time (ms)								
GP	23	Baseline	126.8	7.14				
GP	23	4 months	124.8	7.74			(1,45) = 0.830	0.376
GP	23	6 months	126.4	6.53			(1,45) = 0.046	0.830
GC	24	Baseline	128.5	7.45	(1,46) = 0.617	0.436		
GC	24	4 months	120.4	7.41			(1,47) = 14.2	<0.001
GC	24	6 months	124.8	7.33			(1,47) = 3.09	0.0854
VEP N75-P100 amplitude (microV)								
GP	23	Baseline	6.33	1.74				
GP	23	4 months	6.68	2.46			(1,45) = 0.299	0.587
GP	23	6 months	6.35	2.16			(1,45) = 0.001	0.976
GC	24	Baseline	6.83	2.50	(1,46) = 0.623	0.434		
GC	24	4 months	9.78	2.62			(1,47) = 15.9	<0.001
GC	24	6 months	7.47	2.36			(1,47) = 0.823	0.369

ANOVA, Statistical evaluation by a one-way analysis of variance: GC versus GP eyes at baseline condition; GC and GP eyes at different times of evaluation (4 and 6 months) with respect to the baseline condition

In order to discriminate OAG eyes featuring a potential effect on the RGC function, we correlated the PERG amplitude differences with several parameters (age, time elapsed from the diagnosis of OAG, IOP at the time of the first diagnosis of ocular hypertension, IOP at the time of electrophysiological examination, MD, and PSD), and none of these correlations reached a statistically significant level, thereby leading to the conclusion that after topical citicoline treatment, none of these parameters influences the improvement of the retinal function. By contrast, a significant correlation was found between the values of the PERG amplitude at baseline and the relative differences (4 months minus baseline), thus suggesting that patients who "better responded" to topical citicoline treatment were those that displayed the greater retinal dysfunction at baseline.

It is also known that an improvement of PERG responses may be obtained by lowering the intraocular pressure [17–22]. During the entire period of treatment, considering the IOP values detected in the entire cohort of GC patients (also including the only eye excluded during the follow up for the increase in IOP at 23 mmHg), non-significant ($p>0.01$) changes in IOP were found. Therefore, it can be excluded that the above reported ganglion cell function improvement is due to IOP changes.

As previously reported [26–28], citicoline wash-out induces loss of the acquired improvement of the retinal function and this should be considered as proof of evidence of the efficacy of the treatment.

Neural conduction along the visual pathways (VEP data)

In OAG eyes treated with topical citicoline during a 4-month period, with respect to baseline, we observed a statistically significant improvement of the neural conduction along the visual pathways as suggested by the increase of VEP amplitudes and by the shortening of VEP implicit times. This is in agreement with our previous studies performed through the use of oral or intramuscular citicoline [26–28].

Glaucomatous VEP abnormalities have been ascribed to a dysfunction of the innermost retinal layers (ganglion cells and their fibers) associated with a delay in neural conduction along post-retinal visual pathways [11]. In order to explain the

influence of topical citicoline on VEP responses, the effects on retinal function and on neural conduction along the post-retinal visual pathways may be considered separately. The first was discussed above, whereas the latter is clarified as follows.

Regarding the neural conduction along post-retinal visual pathways, the morpho-functional changes occurring at the dorsolateral geniculate nucleus (dLGN) level in OAG patients need to be considered. In fact, structural and functional damage of the dLGN of human subjects or animals affected by well documented glaucomatous optic neuropathy has been reported [64–66]. Impairment at the dLGN level could cause functional changes in those elements producing visual cortical evoked responses; this is likely to be related to the delay of VEP implicit time observed in OAG patients [11, 67].

When we used citicoline through oral or intramuscular treatment [26–28] we found that this type of administration induced a VEP improvement, which was ascribed (as well as the improvement of the RGC function described above) to a neuromodulator effect of citicoline ("dopaminergic-like activity") at the LGN level. The "dopaminergic-like activity" was considered on the basis of a similar increase in visual function obtained in amblyopic subjects by using intramuscular or oral citicoline [32, 33] or levodopa [68–70] and in consideration of studies performed in patients with Parkinson's disease where

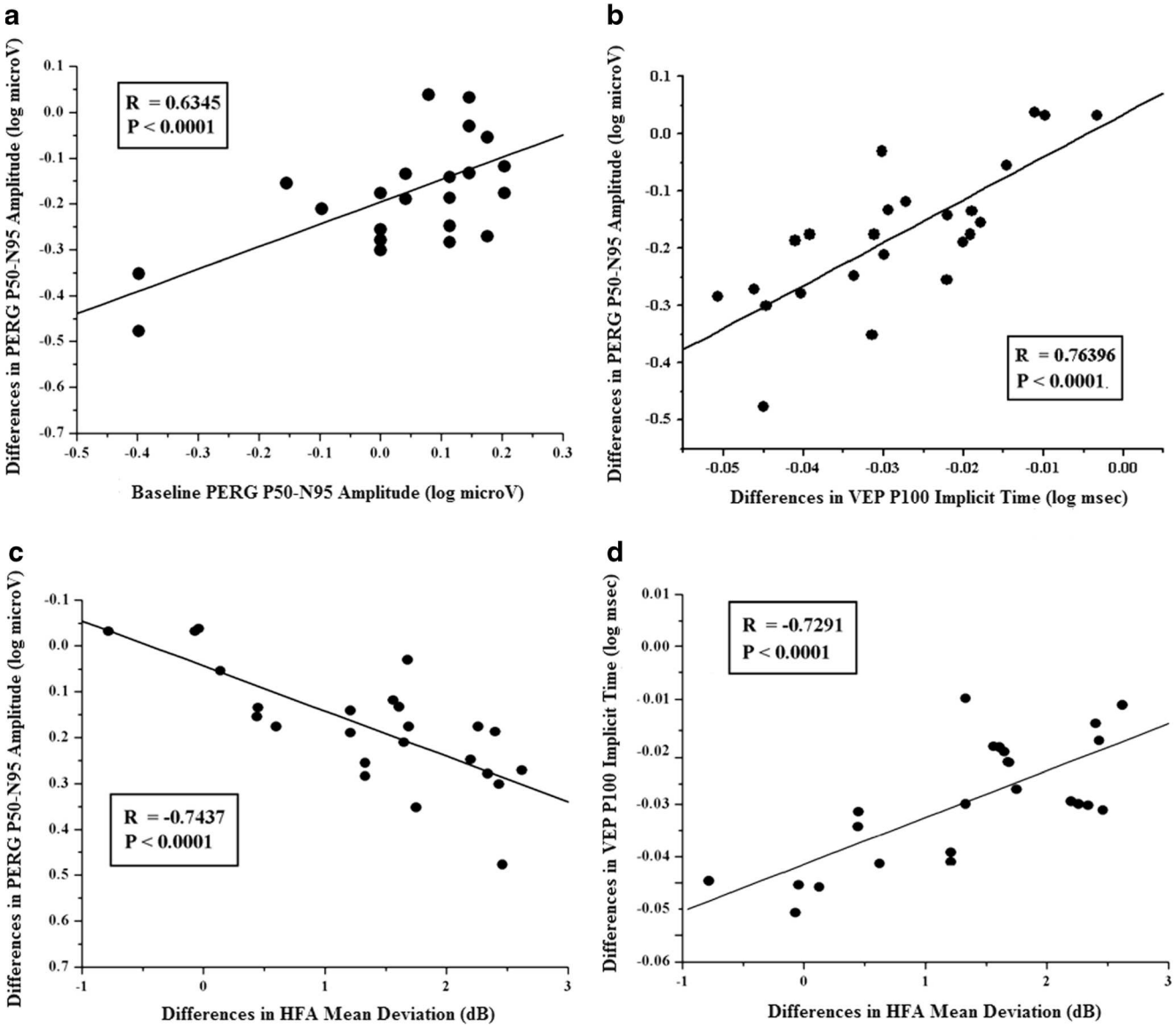

Fig. 4 Individual differences (4 months of treatment minus baseline) of PERG P50-N95 amplitude observed in OAG patients treated with beta-blocker monotherapy plus citicoline plotted as a function of (**a**) baseline PERG P50-N95 amplitude, **b** corresponding differences of VEP P100 implicit time, **c** corresponding differences of Humphrey Field Analyzer Mean Deviation and (**d**) individual differences of VEP P100 implicit time plotted as a function of corresponding differences of Humphrey Field Analyzer Mean Deviation. Pearson's test was used for regression analysis and correlations

the use of citicoline was recommended as a complement to levodopa therapy [71].

In the present study, it was particularly interesting to observe a significant correlation (see Fig. 4) between the increase of PERG amplitude and the reduction of VEP P100 implicit time detected after topical treatment with citicoline. This leads us to believe that a better RGC function may improve the neural conduction along the visual pathways. At the moment, we are not able to document whether citicoline administered by drops, may travel along the RGC axons and reach the LGN with the consequent "dopaminergic-like activity" mentioned above. Thus, the real hypothesis remains whether the enhancement of neural conduction in the visual pathways (improved VEP) is related to the increase of the retinal bioelectrical activity (improved PERG).

Our previous studies, performed through the use of oral or intramuscular citicoline [26–28], found that a period of citicoline wash-out induced a reduction of the acquired enhancement of the neural conduction along the visual pathways obtained after the treatment. Even in this case, the worsening effect after a period of wash-out should be considered as evidence of the usefulness of the treatment.

Conclusions

Our results suggest that topical citicoline in OAG patients may induce an enhancement in the glaucomatous retinal function (PERG improvement) with a consequent better neural conduction along the visual pathways (VEP improvement).

An important question may arise on the concentration of citicoline at the retinal level after the administration of eye drops. This is actually unidentified in humans, but it is be useful to know that this study was designed on the real hypothesis that citicoline may act directly on the structures close to the posterior chamber, since the HPLC assessment showed the presence of citicoline in the vitreous [35]. Indirectly, our PERG results suggest that citicoline administered through topical treatment may have an effect on the retinal structures with the consequent beneficial observed effects.

Another question is related to the age of the cohort of OAG enrolled patients, whose age between 42 and 60 years cannot be considered very old. In view of all that may influence the electrophysiological responses (i.e., cataract or maculopathy, see inclusion criteria), we believe that a similar study performed in older patients may present several confounding factors. At present, we are not able to determine whether citicoline would produce the same observed effects in an older population of OAG patients.

Since we did not intentionally provide adequate statistical analyses about visual field changes, a very important point to clarify lies in establishing whether the retinal and visual pathways enhancement observed after treatment with topical citicoline may produce a stabilization of the glaucomatous visual field defects. To elucidate this point, we believe that a longer follow-up period than the one used for our study (i.e., only 4 months of treatment) together with an adequate statistical number of OAG studied eyes would be necessary. Nevertheless, it was interesting to observe that in several OAG eyes submitted to a short-term treatment with topical citicoline there was an increase in MD related to the improvement of both retinal function (increase in PERG P50-N95 amplitude) and neural conduction along the visual pathways (shortening in VEP P100 implicit time).

Our results could encourage further investigations about the possible visual field and retinal morphology (i.e., by OCT evaluation) changes after treatment with topical citicoline.

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Conflict of interest All authors certify that they have non-financial interest in the subject matter or materials discussed in this manuscript.

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