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Electrophysiological assessment of glaucomatous visual dysfunction during treatment with cytidine-5′-diphosphocholine (citicoline): a study of 8 years of follow-up

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Abstract

In this study we assessed, by simultaneous recordings of visual evoked potentials (VEPs) and pattern-electroretinograms (PERGs), the effects cytidine-5′-diphosphocholine (citicoline) on retinal function and/or visual cortical responses in glaucoma patients. Thirty glaucoma patients were randomly divided into two age-matched groups: patients in group GC (15 patients) were treated with citicoline (1000 mg/die intramuscularly) for 2 months; patients in group GP (15 patients) were treated with placebo for 2 months. After 4 months of wash-out (month 6), GC patients underwent a further 2-month period of citicoline treatment (months 7–8) followed by another 4-month period of wash-out (months 9–12). In GP patients the wash-out was extended for a further 6 months (months 7–12). During the following 13–96 months, GC patients received additional 2-month periods of treatment with citicoline (each period followed by 4 months of wash-out) for a total of 16 periods in 8 years. GP patients were also examined at months 24, 26, 48, 60, 72, 84 and 96. In GC patients the first two treatments with citicoline induced a significant ($p < 0.01$) improvement of VEP and PERG parameters with respect to pre-treatment conditions. VEPs and PERGs recorded in GC patients after the first wash-out revealed that, although there was a worsening trend, the electrophysiological improvement was still maintained with respect to baseline conditions. The additional periods of citicoline treatment in GC patients during the subsequent 13–96 months induced a greater ($p < 0.01$) improvement of VEP and PERG parameters with respect to pre-treatment conditions and when compared to GP patients. Thus, we observed that citicoline significantly improves retinal and cortical bioelectrical responses in glaucoma patients, suggesting a potential use of this substance in the medical treatment of glaucoma, as a complement to hypotensive therapy.

Introduction

It is known that patients affected by open-angle glaucoma (POAG) develop a visual dysfunction that can be revealed by psychophysical methods such as visual field analysis [1, 2], colour vision [3] and contrast sensitivity [4–6]. The visual impairment develops together with clinical signs such as ocular hypertension (intraocular pressure, IOP, >21 mmHg) and characteristic optic nerve head cupping.

In the management of glaucoma, an important goal of ophthalmologists is represented by the possibility of influencing visual function. In this regard, Pecori Giraldi et al. [7] suggested the use of cytidine-5-diphosphocholine (CDP-Choline or citicoline) to improve glaucomatous visual field defects. They observed that 75% of POAG showed a better perimetric condition after treatment with citicoline [7].

The author states that he has no proprietary interest in the development or marketing of this or a competing drug.

Even though perimetric analysis gives a psychophysical assessment of visual function, it has been observed that citicoline increases the level of consciousness [8–12 and see 13 for a review], and thus it was unclear whether the observed changes in glaucomatous visual field [7] could be ascribed to a better performance during the visual field examination and/or to therapeutic effects on impaired retinal and postretinal visual structures.

It was recently suggested that glaucomatous visual field defects might be ascribed to two sources of functional impairment. One, at the retinal level, can be revealed by impaired pattern-ERG (PERG) responses that reflect the bioelectrical activity of ganglion cells and their fibers [14–17], and the other, at the postretinal level, can be revealed by abnormal visual evoked potential (VEP) responses and by a delay in 'retinocortical time' (RCT) [15–17]. RCT represents an index of neural conduction in postretinal visual pathways, derived by simultaneous recordings of VEPs and PERGs [18]. Indeed, a postsynaptic degeneration at the level of the lateral geniculate nucleus (LGN) was suggested [19–23].

Since 1994 we studied the effects of citicoline on glaucomatous retinal and postretinal visual structures by electrophysiological examinations (PERG and VEP) and we found that a 2-month period of treatment with citicoline may induce improvement in both ganglion cell function (PERGs with increase in amplitudes and shortening in times-to-peak) and in neural conduction along postretinal visual pathways (VEPs with increase in amplitudes and shortening in times-to-peak and reduced RCT) [24]. The effects of citicoline on glaucomatous retinal and postretinal structures were not present 8 months after the end of treatment [24]. However, a second 2-month period of treatment with citicoline induced an additional improvement of the glaucomatous retinal and postretinal impairment [24].

In this paper we describe data from our previous study [24] regarding the effects of two periods of citicoline treatment and, since citicoline treatment was continued for the following 13–96 months using the same therapeutic protocol (see below), we present the data of the effects on retinal (PERG) and cortical (VEP) bioelectrical responses obtained during 14 additional periods of treatment. Thus, we present data regarding 8 years of follow-up and the use of PERGs and VEPs in monitoring the therapeutic effects of citicoline on the glaucomatous visual dysfunction.

Materials and methods

Thirty volunteer patients with open-angle glaucoma (OAG) took part in our study.

At the time of diagnosis of glaucoma, IOP was greater than 21 mmHg in the absence of topical treatment in all patients (range 23–27, mean 25.10 ± 1.55 mmHg). All patients thus received topical monotherapy with beta-blockers, inducing an IOP less than 21 mmHg, that remained stable throughout the study (mean 17.5 ± 1.3 mmHg). None of the patients enrolled were subjected to filtration surgery. Other inclusion criteria were: glaucomatous optic nerve head cupping (cup/disc ratio > 0.5), glaucomatous visual field defects (Humphrey 24–2 perimetry–HFA- with mean deviation (MD) between -3 and -6 dB), best corrected visual acuity of 20/20 or better; mean refractive error, when present, between -0.50 and $+0.50$ spherical equivalent; no other ocular, neurological or systemic diseases. None of the patients underwent systemic pharmacological therapy that could potentially influence retinal function and/or neural conduction along visual pathways. Mean age was 45.6 ± 4.3 years.

The 30 patients with glaucoma were randomly divided into groups on the basis of age and MD in order to obtain two age-matched groups: 15 patients were treated with citicoline (GC, 15 eyes) and 15 patients were treated with placebo for the first period of treatment (GP, 15 eyes). No differences in IOP or MD measurements were found between GC and GP patients (GC: 17.4 ± 1.3 mmHg; GP: 17.5 ± 1.5 mmHg).

During the entire study period (96 months, see below), three GP patients and three GC patients were considered as 'drop-out' patients, since they received additional medical treatment or they showed an increase in IOP requiring other topical treatment. We therefore considered 12 GP and 12 GC patients for all statistical analyses.

Informed consent was obtained from each patient enrolled in the study and the research followed the tenets of the Declaration of Helsinki.

The study was approved by the local Ethics Committee.

Pharmacological treatment

Pharmacological treatment was performed according to the following schedule. The results of the 1st and 2nd periods of treatment were reported in our previously published study [24].

1st period
A daily i.m. dose of 1000 mg citicoline (Neuroton®, Nuovo Consorzio Sanitario, Rome, Italy) or placebo (physiological solution with additives) was prescribed according to the following protocol:

– 0–2 months: first period of pharmacological treatment with citicoline or placebo;
– 3–6 months: first period of wash-out and follow-up at the 6th month.

Each GC or GP patient received 20 unlabeled boxes with three vials each, for a total of 60 vials. The vials contained citicoline (for GC patients) or placebo (for GP patients). In order to perform a double-blind study, the boxes were numbered by Nuovo Consorzio Sanitario, who held the key, and patients were tested by V.P., who was unaware of the contents of the vials.

2nd period
When the key was opened, and we observed the worsening trend of electrophysiological parameters (see below: results at month 6), we decided to subject GC patients to a second period of two months (months 7–8) of pharmacological treatment with citicoline (each patient received an additional series of 20 unlabeled boxes with three vials each of citicoline, for a total of 60 vials) followed by a second wash-out period of 4 months (months 9–12); follow-up was at 12 months. In GP patients the wash-out period was extended for a further 6 months.

Subsequent periods
Considering the positive effect on retinal and cortical responses, GP patients received a daily i.m. dose of 1000 mg citicoline (now, Cebroton 1000®, Tubilux Pharma, Pomezia, Italy) for a further 2-month period followed by 4 months of wash-out.

This therapeutic protocol (2-month period of treatment with citicoline followed by 4 months of wash-out) was repeated for a total period of 96 months (8 years).

Thus, additional treatments were performed during months 13–14, 19–20, 25–26, 31–32, 37–38, 43–44, 49–50, 55–56, 61–62, 67–68, 73–74, 79–80, 85–86, 91–92; and the additional wash-out periods were carried out during months 15–18, 21–24, 27–30, 33–36, 39–42, 45–48, 51–54, 57–60, 63–66, 69–72, 75–78, 81–84, 85–90, 93–96.

Electrophysiological assessment

In GC patients, simultaneous recordings of VEPs and PERGs were assessed at baseline conditions (day 0), at the end of each 2-month period of treatment with citicoline (months 2, 8, 14, 20, 26, 32, 38, 44, 50, 56, 62, 68, 74, 80, 86, 92), and at the end of the 4-month periods of wash-out (months 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96).

In GP patients, simultaneous recordings of VEPs and PERGs were assessed at baseline conditions (day 0), after the first 2-month period of treatment with placebo (months 2), after the first period of wash-out (month 6), after another 2 and 6 months (month 8 and 12) and every 12 months during the subsequent 8 years (months 24, 36, 48, 60, 72, 84, 96).

All electrophysiological examinations were performed using a previously published method [15–17, 24].

Briefly, the subjects under examination were seated in an acoustically isolated semi-dark room in front of a display surrounded by a uniform field (120×120 degree) of luminance (5 cd/m^2). Subjects had been previously informed of the procedure and its diagnostic uses.

Prior to the experiment, each subject was adapted to the ambient room in front of the visual stimuli (see below) for 10 min and, since a little miosis occurred, pupil diameter was approximately 5 mm. Miotic or mydriatic drugs were never used.

The visual stimuli were checkerboard patterns (contrast, expressed as $L_{max} - L_{min} / L_{min} + L_{max,}$ was 95%; mean luminance 100 cd/m^2) generated on a TV monitor and reversed in contrast at the

rate of 2 reversal/s. At the viewing distance of 114 cm the check edges subtended 15 min of visual angle and the screen of the monitor subtended 12.5 degrees. The refraction of all subjects was corrected for the viewing distance. Stimulation was monocular, after occlusion of the other eye.

VEP recordings

Ag/AgCl cup-shaped electrodes were fixed with collodion in the following positions: active electrode in Oz, reference electrode in Fpz, (EEG International System 10–20, [25]), ground on the left arm.

Interelectrode resistance was kept below 3 kΩ. The bioelectric signal was amplified (gain 20,000), filtered (band-pass 1–100 Hz) and averaged (200 artefact-free events were averaged for each trial) by BM 6000. Analysis time was 250 m.

The transient VEP was characterized by several waves with three peaks, which in normal subjects and in our experimental conditions, appeared after 75, 100 and 145 ms. These peaks had negative (N75), positive (P100) and negative (N145) polarity, respectively.

PERG recordings

The bioelectrical signal was recorded by means of Ag/AgCl small cup-shaped electrodes placed on the inferior eyelid. A discussion comparing PERG responses obtained by skin electrodes to the responses obtained by corneal electrodes can be found elsewhere [26, 27]. Monocular electroretinograms were derived bipolarly between the tested (active electrode) and the patched (reference electrode) eye using the method described by Fiorentini et al. [28]. The ground electrode was on Fpz. Interelectrode resistance was maintained below 3 kΩ. The signal was amplified (gain 50,000), filtered (band pass 1–30 Hz) and averaged with automatic rejection of artefacts (200 artefact-free events were averaged for each trial) by BM 6000 (Biomedica Mangoni, Pisa, Italy). Analysis time was 250 ms.

The transient PERG was characterized by several waves with three peaks, which in normal subjects and in our experimental conditions, appeared after 35, 50 and 95 ms. These peaks had negative (N35), positive (P50) and negative (N95) polarity, respectively.

During the recording session, VEPs and PERGs were simultaneously recorded at least twice and the resulting waveforms were superimposed to check the repeatability of the results.

We accepted VEP and PERG signals with signal-to-noise ratio >2. The noise was measured by recording bioelectrical signals while the monitor was screened by a cardboard, and a noise <0.1 μV (mean 0.085 μV) was observed in all subjects tested.

For all VEPs and PERGs, times-to-peak and peak amplitudes of each wave were directly measured on the displayed records by means of a pair of cursors. Simultaneous recordings of VEPs and PERGs allowed us to derive retinocortical time (RCT) as the difference between VEP P100 and PERG P50 times-to-peak [18].

On the basis of our previously published results, reporting a significant improvement of PERG and VEP parameters after treatment with citicoline [24] and since after the wash-out period there was a reduction of this improvement [24], in the present study we concentrated on the long-term effects of citicoline treatment, evaluating the differences observed at the end of each period of follow-up with respect to baseline conditions. These differences were evaluated by one-way analysis of variance (ANOVA). Pearson's correlation was used to correlate MD of Humphrey 24–2 visual field with all electrophysiological parameters. PERG and VEP times-to-peak and amplitudes, and RCT data underwent logarithmic transformation to better approximate a normal distribution. In all analyses, a p value less than 0.01 was considered statistically significant.

Results

Examples of simultaneous VEP and PERG recordings performed in one glaucoma patient at baseline conditions, after each period of treatment with citicoline and after each period of wash-out are displayed in Figure 1. Examples of HFA 24–2 performed in one GC patient and in one GP eye at baseline conditions and after 96 months are shown in Figure 2. The correlation between electrophysiological parameters and MD of HFA 24–2 observed in all glaucoma patients at baseline conditions is shown in Figure 3.

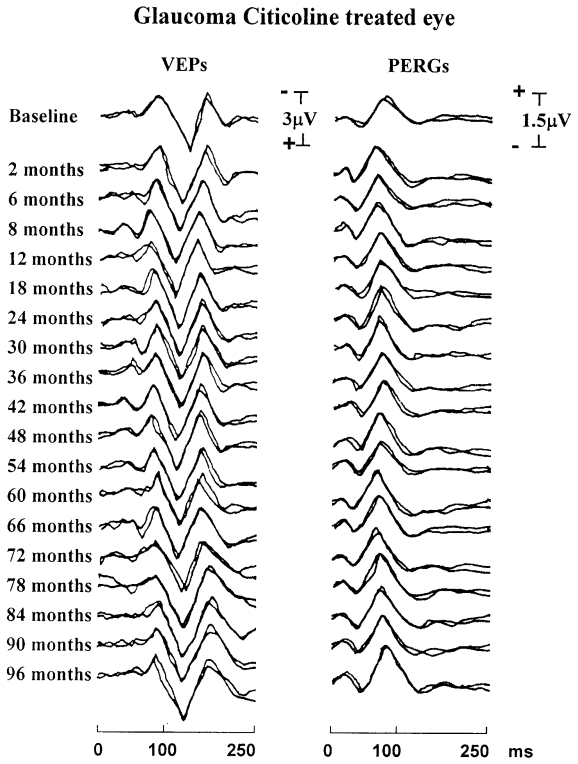

Figure 1. Layout of simultaneous VEP and PERG recordings in one glaucoma patient treated with citicoline (GC). Electrophysiological examinations were assessed at baseline conditions and at 2, 6, 8, and 12 months after medical treatment with citicoline. Citicoline treatment was performed in two different 2-month periods (0–2 and 7–8 months) followed by two 4-month periods of wash-out (3–6 and 9–12 months). The GC patient received additional 2-month periods of citicoline treatment and each period was followed by a 4-month period of wash-out for a total of 14 periods during the subsequent 7 years. We concentrated on the long-term effects of citicoline treatment and we therefore reported VEP and PERG recordings assessed at the end of each wash-out period: 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, and 96 months. PERGs and VEPs recorded after citicoline treatment showed a decrease in times-to-peak and increase in amplitudes when compared to baseline conditions.

Mean data and statistical analyses are shown in Figures 4–8.

At baseline conditions, similar values of VEP and PERG parameters ($p > 0.05$) were observed in GC and GP patients (see Figures 2–6 'month 0').

The results of the 1st and 2nd period of evaluation are in agreement with the results of our previously published study [24].

Briefly, GP patients displayed similar VEP and PERG parameters in all examinations performed. In GC patients, on the other hand, after the first period of treatment (months 0–2) with citicoline we observed a significant ($p < 0.01$) shortening in VEP P100 and PERG P50 times-to-peak, a significant increase in VEP N75-P100 and PERG P50-N95 amplitudes and a significant reduction of RCT. VEPs and PERGs, recorded in GC patients after 4 months of wash-out (month 6), revealed that, although there was a worsening trend, the electrophysiological improvement with respect to baseline conditions was still maintained. The second 2-month period of treatment with citicoline (months 7–8) induced a further ($p < 0.01$) improvement of VEP and PERG parameters. After the second 4-month period of wash-out (month 12), VEP N75-P100 amplitudes were still significantly increased when compared to baseline; VEP P100 and PERG P50 times-to-peak and RCTs were still significantly reduced with respect to baseline values; PERG P50-N95 amplitudes were similar to baseline conditions.

Subsequent evaluations

In GC patients, there was an increase in VEP N75-P100 and PERG P50-N95 amplitudes, a decrease in VEP P100 and PERG P50 times-to-peak, and a decrease in RCT after each period of treatment with respect to pre-treatment values. After each wash-out period, GC patients showed a decrease in VEP N75-P100 and PERG P50-N95 amplitudes, an increase in VEP P100 and PERG P50 times-to-peak, and an increase in RCTs with respect to the values observed at the end of treatment. However, it is worth noting that after each wash-out period VEP N75-P100 and PERG P50-N95 amplitudes were still significantly ($p < 0.01$) higher and VEP P100 and PERG P50 times-to-peak and RCTs were still significantly ($p < 0.01$) shorter in GC patients when compared to baseline conditions and when compared to GP patients.

At the end of follow-up (96 months), an increase in MD of HFA 24–2, with respect to baseline values, was also observed in all GC patients. This increase was significantly related to the increase in VEP N75-P100 and PERG P50-N95 amplitudes, to the decrease in VEP P100 and PERG P50 times-to-peak, and to the decrease in RCT with respect to pre-treatment values. The correlations are shown in Figure 9.

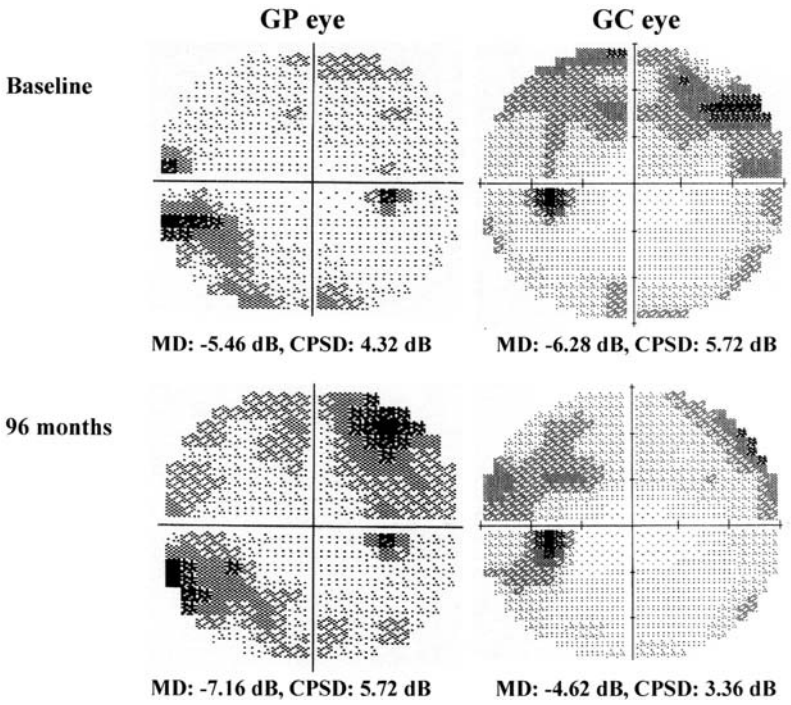

Figure 2. Examples of HFA-24 assessed at baseline conditions and after 96 months in one OAG eye subjected to beta-blocker therapy alone (GP eye) and in one OAG eye subjected to beta-blocker therapy plus citicoline (GC eye). With respect to baseline conditions, it is possible to observe a worsening of the visual field in the GP eye, while in the GC eye an improvement of the visual field can be detected.

During the whole period of treatment, none of the patients reported adverse side effects. There were no significant changes in intraocular pressure in any of the subjects tested.

Discussion

The use of electrophysiological tests (PERG and VEP) to detect human glaucomatous visual dysfunctions is widely documented in several studies [29–49]. It is known that electrophysiological tests may represent objective methods with less intra-individual variability than visual field analysis, supporting the use of PERGs and VEPs in monitoring the glaucomatous dysfunction [37, 41, 43].

In the present study we used repeated assessments of simultaneous recordings of VEPs and PERGs to evaluate changes of retinal and visual cortical responses in patients with glaucoma after treatment with citicoline.

In our glaucomatous patients, treatment with citicoline induces an improvement of retinal bioelectric responses (PERGs with increase in amplitudes and shortening in times-to-peak), of visual cortical bioelectric responses (VEPs with increase in amplitudes and shortening in times-to-peak) and an improvement of the index of neural conduction in postretinal visual pathways (reduced RCT).

Our previous results [24] showed that at 8 months after suspension of treatment with citicoline, it is not possible to observe any therapeutic effect on the function of the retina and visual pathways, and thus the positive effects cease with the interruption of drug administration. On the other hand, in those patients in which repeated treatments with citicoline were performed, a stable improvement of the function of the retina and visual pathway was observed during the entire 8-year follow-up period with respect to pre-treatment (baseline) conditions.

Although our results clearly suggest a positive effect of citicoline in reducing the retinal and postretinal glaucomatous dysfunction, the mechanism of action of citicoline in the visual system is not entirely understood.

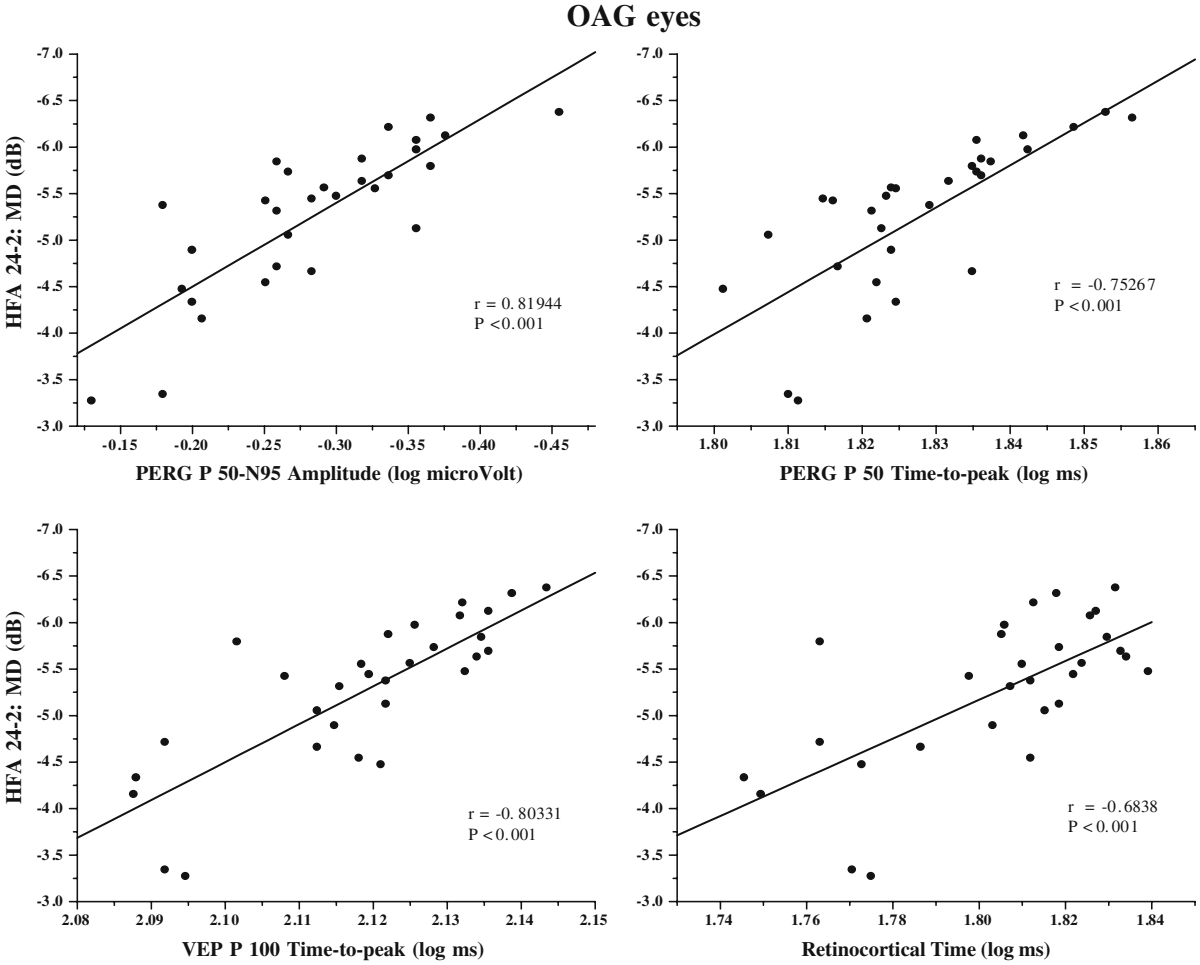

Figure 3. Baseline conditions. PERG P50-N95 amplitude, PERG P50 Time-to-peak, VEP P100 Time-to-peak and RCT observed in all OAG patients enrolled in the study plotted against MD of Humphrey 24–2 visual field analysis. Pearson's test was used for regression analysis.

Indeed, possible mechanisms of action of citicoline have been widely discussed in our previous report [24]. Briefly, citicoline is an endogenous substance that acts as intermediary in the synthesis of phosphatidylcholine, (a major phospholipid in the neuronal membrane [50–53]) through the activation of the biosynthesis of structural phospholipids in neuronal membranes. Citicoline increases the metabolism of cerebral structures [52] and inhibits phospholipid degradation [53]. It may therefore have potential neuroprotective and neuromodulator roles as demonstrated in conditions of cerebral hypoxia and ischemia [52, 53] and by the evidence of induced increase in the levels of different neurotransmitters and neuromodulators, including noradrenaline, in the central nervous system. In addition, several studies suggest that citicoline successfully increases the level of consciousness in different brain disorders ascribed to vascular, traumatic or degenerative processes [8–12 and see 13 for review].

This study began with the aim of evaluating if a possible increase in levels of consciousness could explain the improved psychophysical responses, evaluated by visual field analysis, observed in glaucomatous patients after treatment with citicoline [7].

The results obtained by PERG and VEP analysis suggested that citicoline, apart from increased levels of consciousness, may improve retinal function and neural conduction at the postretinal level.

Glaucomatous retinal dysfunctions were assessed by PERG recordings. Given that in

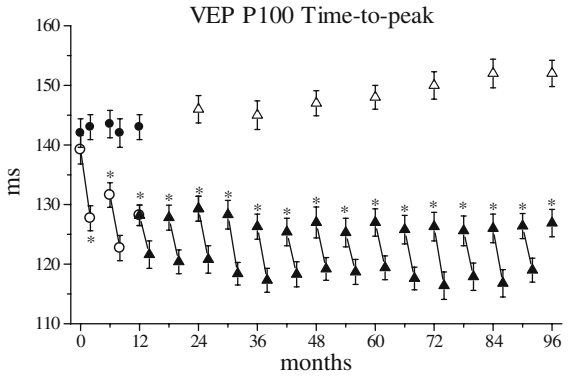

Figure 4. Graphic representation of mean values of VEP P100 peak latencies observed in glaucoma patients at baseline conditions and after medical treatment with placebo (GP, ●) or citicoline (GC, **O**). Medical treatment with placebo or citicoline was performed over a 2-month period (0–2 months) followed by a 4-month period of wash-out (3–6 months). At month 6, GC patients underwent a second 2-month period of citicoline treatment (**O**, 7–8 months) followed by a second 4-month period of wash-out (9–12 months). In GP patients, after the first 6 months, the period of wash-out was extended for a further 6 months and VEPs were also recorded at 8 and 12 months. GC patients received additional 2-month periods of citicoline treatment (▲) and each period was followed by 4 months of wash-out for a total of 14 periods in the subsequent 7 years. GP patients were further examined at months 24, 26, 48, 60, 72, 84 and 96 (△). The solid line within **O** and ▲ indicate the periods of treatment. The absence of a line within **O** and ▲ indicates the wash-out periods. Vertical lines represent one standard error of the mean. We concentrated on the long-term effects of citicoline treatment by comparing the differences observed at the end of each period of wash-out with respect to baseline conditions, by ANOVA. *: $p < 0.01$ versus baseline and GP.

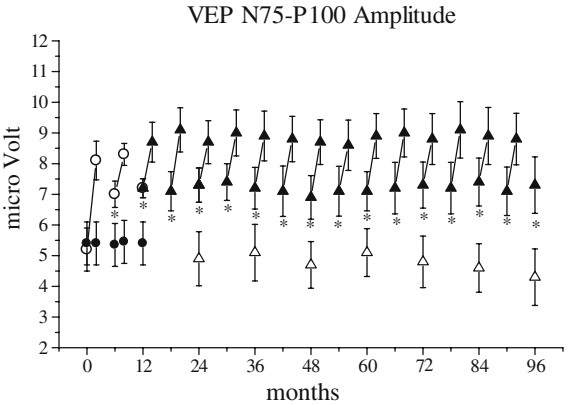

Figure 5. Graphic representation of mean values of VEP N75-P100 peak amplitude observed in glaucoma patients treated with placebo or without treatment (GP: ●, △) or treated with citicoline (GC: **O**; ▲, solid line). The absence of a line within **O** and ▲ indicates the wash-out periods. Vertical lines represent one standard error of the mean. ANOVA versus baseline and GP, *: $p < 0.01$.

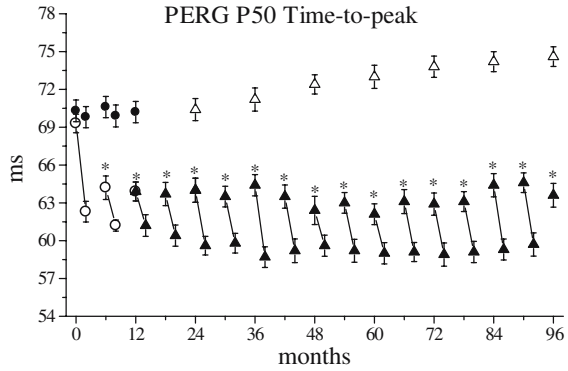

Figure 6. Graphic representation of mean values of PERG P50 peak latency observed in glaucoma patients treated with placebo or without treatment (●, △) or treated with citicoline (**O**; ▲, solid line). The absence of a line within **O** and ▲ indicates the wash-out periods. Vertical lines represent one standard error of the mean. ANOVA versus baseline and GP, *: $p < 0.01$.

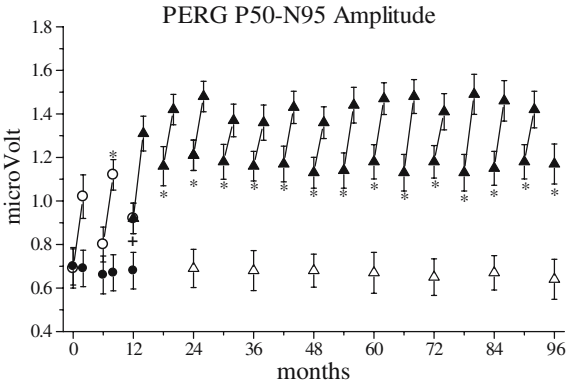

Figure 7. Graphic representation of mean values of PERG P50-N95 peak amplitude observed in glaucoma patients treated with placebo or without treatment (●, △) or treated with citicoline (**O**; ▲, solid line). The absence of a line within **O** and ▲ indicates the wash-out periods. Vertical lines represent one standard error of the mean. ANOVA versus baseline and GP, *: $p < 0.01$; +: $p > 0.01$.

glaucoma a loss of ganglion cells and their fibers has been documented by histological studies [54–56] and by objective methods of *in vivo* morphological evaluation of retinal fibers [17, 57–59], the impaired PERG responses observed in patients with glaucoma could be ascribed to a dysfunction of the innermost retinal layers [15–17, 29–41], although a functional impairment of preganglionic elements has been also suggested [60–62].

The improvement of PERG responses after treatment was ascribed to a dopaminergic-like activity of citicoline [24]; in fact, levodopa was found to increase retinal function in humans

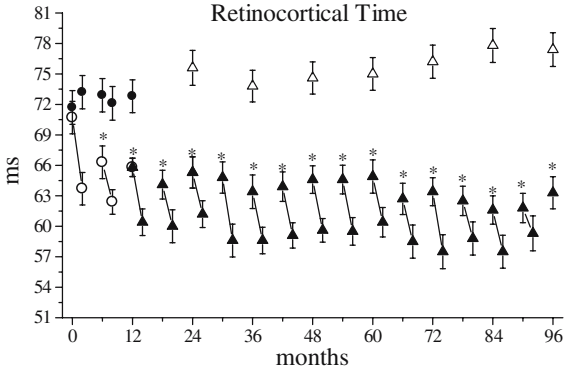

Figure 8. Graphic representation of mean values of RCT, difference between VEP P100 and PERG P50 time-to peak, an electrophysiological index of neural conduction along postretinal visual pathways) observed in glaucoma patients after medical treatment with placebo or without treatment (●, △) or treated with citicoline (**O**; ▲, solid line). The absence of a line within **O** and ▲ indicates the wash-out periods. Vertical lines represent one standard error of the mean. ANOVA versus baseline and GP, *: $p < 0.01$.

treated with this substance [63], and our results could therefore be explained by a similar neuromodulator activity.

In our study we did not perform any morphological examination, and thus, although our results indicate that citicoline improves the bioelectrical retinal activity, we were not able to demonstrate whether there were other effects on retinal fibers (i.e., an increase in retinal nerve fiber layer thickness).

The results obtained in our glaucoma patients indicate the treatment with citicoline induces an improvement in neural conduction along the visual pathways as suggested by the shortening in RCT and VEP P00 times-to-peak and the increase in VEP N75-P100 amplitudes. Similar results have been recently obtained with oral administration of citicoline [42].

The effects of citicoline in the visual system were revealed by the improvement of visual acuity [51, 64, 65], of VEP responses and contrast sensitivity [66] in amblyopic subjects after treatment with this substance. Since similar results were obtained in amblyopic subjects after treatment with levodopa [67–69], and studies performed in patients with Parkinson's disease recommended the use of citicoline as a complement to levodopa therapy [70–72], a dopaminergic-like activity could once again be suggested to explain VEP and RCT results after treatment with citicoline.

Glaucomatous VEP abnormalities have recently been ascribed to impaired neural conduction along postretinal visual pathways related to a dysfunction of the innermost retinal layers (ganglion cells and their fibers) [15–17]. In order to explain the influence of citicoline in VEP responses, an independent effect on neural conduction in postretinal visual pathways or in visual cortical cells could be also hypothesized. There is, however, no clear or conclusive experimental or published data to support this hypothesis, and the improvement of retinal function lead us to believe that the changes in neural conduction along the visual pathways could be dependent on the reduced dysfunction of the innermost retinal layers.

We have recently observed that the MD of Humphrey perimetry is significantly related to PERG and VEP parameters and to RCT [16] and this is confirmed by the data obtained in our enrolled OAG patients (see Figure 3). Thus, it is likely that the above-mentioned sources of cortical improvement could also be suggested to explain the improvement of perimetric conditions previously reported after treatment with citicoline [7, 73].

At the end of follow-up (96 months), we observed an increase in MD of HFA 24–2 with respect to baseline values in all GC patients. This increase was significantly related to the increase in VEP N75-P100 and PERG P50-N95 amplitudes, to the decrease in VEP P100 and PERG P50 times-to-peak, and to the decrease in RCT with respect to pre-treatment values (see Figure 9.). This lead us to believe that the previously reported changes of perimetric conditions [7, 73] may be ascribed to an improvement of both retinal and post-retinal structures induced by citicoline treatment.

The results observed in our OAG patients subjected to treatment with beta-blockers plus citicoline (GC eyes) compared to OAG patients treated with beta-blockers only (GP eyes, in which there was a worsening of the visual field and of electrophysiological parameters at 96 months) may suggest the potential use of citicoline in order to induce a direct neuroprotective effect aimed towards the stabilization or the improvement (as observed in our GC eyes) of the glaucomatous visual function.

In agreement with the reported observations of similar studies [24, 42, 51–53, 64–66], an important

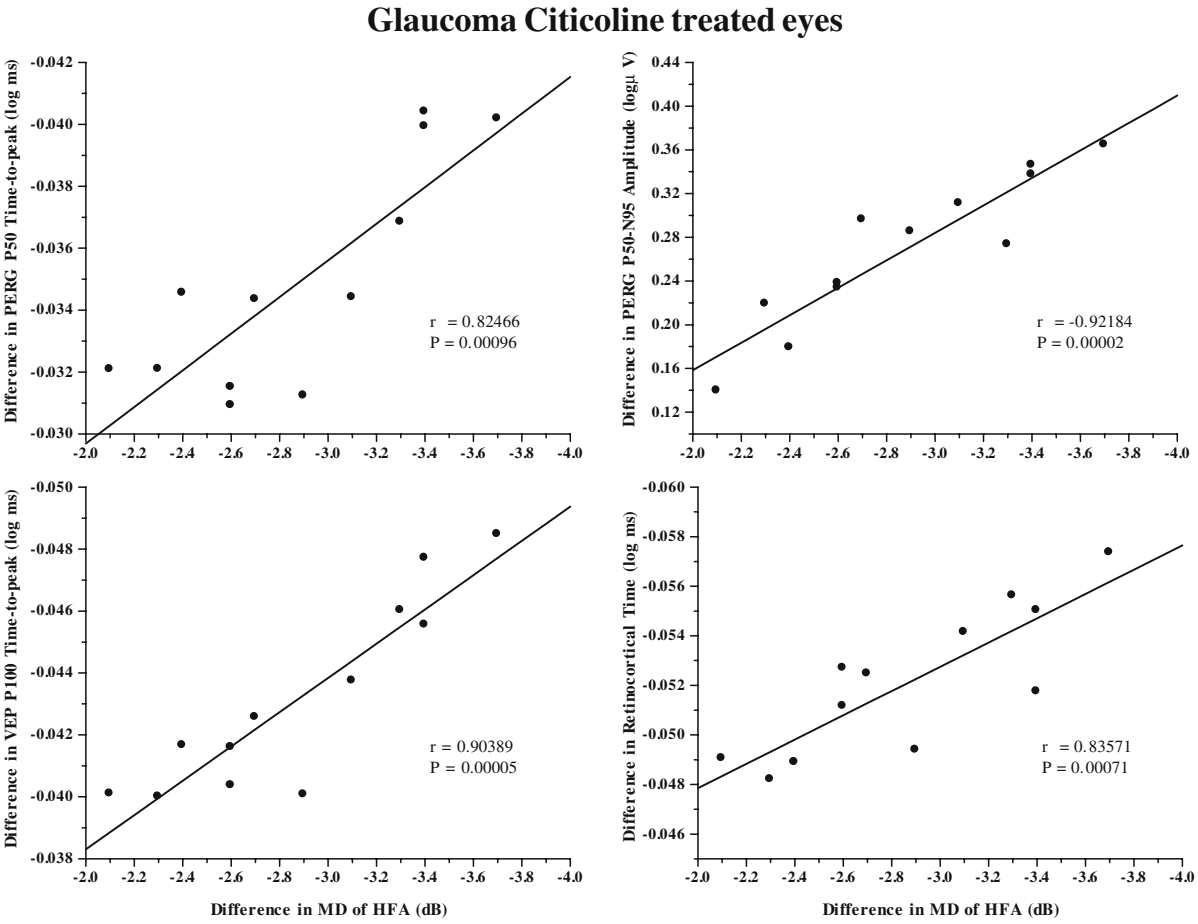

Figure 9. Differences in MD of Humphrey 24–2 visual field analysis observed in individual glaucoma citicoline-treated eyes (GC eyes) at the last follow-up (96 months) with respect to baseline values, plotted against differences in PERG P50-N95 amplitude, PERG P50 Time-to-peak, VEP P100 Time-to-peak and Retinocortical Time. Pearson's test was used for regression analysis.

aspect of this study is the lack of adverse pharmacological side effects in all participating subjects, even after long-term administration of the drug.

In conclusion, our results suggest an important role of electrophysiological tests in the objective evaluation of changes in retinal and cortical responses in glaucomatous patients subjected to pharmacological treatment.

We also report that citicoline significantly improves retinal and cortical responses in glaucoma patients, indicating a potential use of this substance in the medical treatment of glaucoma, as a complement to hypotensive therapy [7, 24, 42, 73].

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