Cytidine-5′-Diphosphocholine (Citicoline) Improves Retinal and Cortical Responses in Patients with Glaucoma

Vincenzo Parisi, MD,[1,2] *Gianluca Manni, MD,*[1] *Gaspare Colacino, MD,*[1] *Massimo G. Bucci, MD*[1]

Purpose: To evaluate the effects of cytidine-5′-diphosphocholine (citicoline) on retinal function and on cortical responses in patients with glaucoma.

Design: Randomized clinical trial.

Participants: Forty patients with open-angle glaucoma were randomly divided into two age-matched groups: citicoline group ([GC] n = 25) and placebo group ([GP] n = 15).

Methods: The GC patients were treated with Neuroton (citicoline, 1000 mg/day intramuscularly) for 60 days; GP patients were treated with placebo (physiologic solution with additives) for 60 days. After 120 days of washout (day 180), the GC patients were divided into two age-matched groups: in 10 patients (GC1 group) the washout was prolonged for a further 120 days; in 15 patients (GC2 group) a second 60-day period of citicoline treatment was followed by a second 120-day period of washout. At day 180, the washout was extended for another 180 days in GP patients. In all subjects, retinal and cortical responses were evaluated by simultaneous recordings of visual evoked potentials (VEPs) and pattern-electroretinograms (PERGs) at baseline, after 60 days, and after 180 days. At day 300, VEPs and PERGs were also evaluated in GC1 patients, and at 240 and 360 days in GC2 and GP patients.

Main Outcome Measures: Visual evoked potential parameters (P100 latency and N75-P100 amplitude); PERG parameters (P50 latency and P50-N95 amplitude); and intraocular pressure.

Results: The GP patients displayed similar VEP and PERG parameters in all examinations performed. In GC patients, the treatment with citicoline induced a significant ($P < 0.01$) improvement of VEP and PERG parameters, and their values were significantly different ($P < 0.01$) with respect to those of GP patients ($P < 0.01$). Visual evoked potentials and PERGs, recorded in GC patients after washout, revealed that although there was a worsening trend, the electrophysiologic improvement was still maintained. After a second period of washout, GC1 patients had VEP and PERG parameters similar ($P > 0.05$) to baseline ones and to those of GP patients. In GC2 patients, a second period of citicoline treatment induced a further ($P < 0.01$) improvement of VEP and PERG parameters

Conclusion: Citicoline may induce an improvement of the retinal and of the visual pathway function in patients with glaucoma. *Ophthalmology 1999;106:1126–1134*

Patients with open-angle glaucoma show an abnormal visual function that develops together with clinical signs such as ocular hypertension (intraocular pressure [IOP] > 21 mmHg) and characteristic optic nerve head cupping. This impairment of visual function may be revealed by psychophysical methods such as visual field analysis,[1,2] color vision,[3] and contrast sensitivity.[4–6]

The possibility of influencing the visual function may be a goal of ophthalmologists in the management of glaucoma. Toward this end, Pecori Giraldi et al[7] suggested a therapeutic effect of cytidine-5-diphosphocholine (CDP-choline, or citicoline) in patients with glaucoma, and they found that 75% of glaucomatous eyes showed a better perimetric condition after treatment with this substance.

Although perimetric analysis gives a psychophysical response, it does not reveal which structures of the visual system may selectively contribute to the improvement of the perimetric condition observed,[7] and therefore electrophysiologic tests appear to be more appropriate methods for assessing the different structures contributing to visual function. In fact, the function of the different retinal layers can be objectively evaluated by recordings of electroretinographic signals evoked by flash (flash ERG) or patterned stimuli (PERG),[8–12] whereas the whole visual pathway

Originally received: September 28, 1998.
Revision accepted: March 8, 1999. Manuscript no. 98652.

[1] Cattedra di Clinica Oculistica, Universita' di Roma Tor Vergata, Rome, Italy, and Fondazione per l'Oftalmologia G.B. Bietti, Rome, Italy.

[2] AFaR. CRCCS, Divisione Oculistica Ospedale Fatebenefratelli, Isola Tiberina, Rome, Italy.

Presented in part at the Association for Research in Vision and Ophthalmology annual meeting, Fort Lauderdale, Florida, May 1998.

The authors have no proprietary interest in the development or marketing of this or a competing drug.

Address correspondence to Vincenzo Parisi, Cattedra di Clinica Oculistica, Universita' di Roma Tor Vergata, Roma, Via Santa Maria Goretti 66, 00199-Rome Italy. E-mail:vparisi@tin.it.

function can be assessed by recordings of cortical responses evoked by patterned stimuli (visual evoked potentials [VEPs]).[13] An index of the neural conduction in the post-retinal visual pathways expressed as "retinocortical time" (RCT) can be derived by simultaneous recordings of VEPs and PERGs.[13,14]

Of note, electrophysiologic tests performed in cats, monkeys, and humans with ocular hypertension or glaucoma showed a normal flash ERG and[15,16] impaired PERGs[15–25] and VEPs.[19,20,26–28] Abnormal flash ERGs were observed in patients with advanced glaucoma,[29] and delayed RCTs were found in glaucoma patients only.[30]

This study was performed to establish, by electrophysiologic methods, what effects citicoline could induce on the retinal function and on the visual cortical responses in glaucoma patients.

Materials and Methods

Forty volunteer patients with open-angle glaucoma took part in the study. In all patients enrolled, when the diagnosis of glaucoma was made, the IOP was greater than 21 mmHg without any topical treatment (range, 23–27 mmHg; mean, 25.10 ± 1.55 mmHg). Each patient received topical treatment with beta-blockers only, and an IOP less than 21 mmHg was observed (mean, 17.5 ± 1.3 mmHg). Filtration surgery had never been performed in any of the patients participating in this study. Other inclusion criteria were glaucomatous optic nerve head cupping (cup:disc ratio > 0.5); glaucomatous visual field defects (Humphrey 24-2 perimetry with mean deviation 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; and no other ocular, neurologic, or systemic disease. The mean age was 45.6 ± 4.3 years.

The 40 patients with glaucoma were randomly divided into two age-matched groups: 25 were treated with citicoline (GC, 25 eyes), and 15 were treated with placebo (GP, 15 eyes). No differences in the IOP measurements were found between GC and GP patients (GC, 17.4 ± 1.3 mmHg; GP, 17.5 ± 1.5 mmHg).

Pharmacologic Treatment

The pharmacologic treatment was performed at two different times:

First Period. A daily intramuscular dose of 1000 mg citicoline (Neuroton, Nuovo Consorzio Sanitario, Rome, Italy) or placebo (physiologic solution with additives) was prescribed following this protocol:

0–60 days: first period of pharmacologic treatment with citicoline or placebo;

61–180 days: first period of washout and follow-up at day 180.

Each GC or GP patient received 20 unlabeled boxes with 3 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 knew the key, and the patients were tested by one examiner (VP), who was unaware of the contents of the vials.

Second Period. When we observed the worsening trend of electrophysiologic parameters (see results at day 180), we decided to randomly divide the GC patients into two age-matched groups:

GC1 group (10 patients, 10 eyes): the period of washout was extended for another 120 days, and the follow-up was assessed at day 300.

GC2 group (15 patients, 15 eyes): a second 60-day period of pharmacologic treatment with citicoline was performed (181–240 days, and each patient received another 20 unlabeled boxes with 3 vials each of citicoline, for a total of 60 vials) followed by a second period of washout (241–360 days); the follow-up was at 360 days.

It is worth noting that for all electrophysiologic parameters, no differences were found between GC1 and GC2 groups (see results at 180 days).

In GP patients, the period of washout was extended for an additional 180 days.

During the entire period of treatment with citicoline or placebo and during the whole washout period for all glaucoma patients, no other general pharmacologic treatments were given, but the topical treatment with beta-blockers was continued.

Informed consent was obtained from each patient enrolled in this study, and the research followed the tenets of the Declaration of Helsinki. The study was previously approved by the local Ethical Committee.

Electrophysiologic Assessment

In GP and GC patients simultaneous recordings of VEP and PERG were assessed at baseline (day 0), after the period of 60 days of treatment with placebo or citicoline (day 60), and after the period of washout (day 180).

Further electrophysiologic evaluations were performed in GC1 patients after the second 120-day period of washout (day 300); in GC2 patients after the second 60-day period of treatment with citicoline (day 240), and after the second 120-day period of washout (day 360). In GP patients, VEP and PERG were also recorded twice during the extended period of washout (days 240 and 360).

The electrophysiologic examinations were performed using a previously published method.[30,31]

The subjects were seated in an acoustically isolated semidark room in front of the display that was surrounded by a uniform field (120- × 120-degree) of luminance of 5 cd/m^2. The subjects were informed of the type of examination and its diagnostic uses.

Prior to the experiment, each subject was adapted to the ambient room in front of the visual stimuli light (see below) for 10 minutes, and because a little miosis occurs, the pupil diameter was about 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 television monitor and reversed in contrast at the rate of 2 reversals per second. At the viewing distance of 114 cm the check edges subtend 15 minutes of visual angle, and the screen of the monitor subtended 12.5 degrees. The refraction of all subjects was corrected for the viewing distance. The stimulation was monocular, after occlusion of the other eye.

Visual Evoked Potential 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[32]), and ground on left arm.

The interelectrode resistance was kept below 3 kohms. The bioelectric signal was amplified (gain 20000), filtered (band-pass 1–100 Hz), and averaged (200 events free from artifacts were averaged for every trial) using the BM 6000 (Biomedica Mangoni, Pisa, Italy). The analysis time was 250 msec.

The transient VEP was characterized by several waves with 3 peaks, which in normal subjects and in our experimental condition

appeared after 75, 100, and 145 ms. These peaks had negative (N75), positive (P100), and negative (N145) polarity, respectively.

Pattern-electroretinogram Recordings. The bioelectric signal was recorded by means of Ag/AgCl small cup-shaped electrodes placed on the inferior eyelid. Monocular electroretinograms were derived bipolarly between the tested (active electrode) and the patched (reference electrode) eye using the method described by Fiorentini et al.[33] The ground electrode was on Fpz. The interelectrode resistance was maintained lower than 3 kohms. The signal was amplified (gain 50000), filtered (band pass 1–30Hz), and averaged with automatic rejection of artifacts (200 events free from artifacts were averaged for every trial) using the BM 6000. The analysis time was 250 ms.

The transient PERG was characterized by several waves with 3 peaks, which in normal subjects and in our experimental condition appeared after 35, 50, and 95 ms. These peaks had negative (N35), positive (P50), and negative (N95) polarity, respectively.

In the recording session, simultaneous VEPs and PERGs were 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 ratios >2. The noise was measured by recording the bioelectric 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, the peak latency and the peak amplitude of each of the waves were measured directly on the displayed records by means of a pair of cursors. Simultaneous recording of VEPs and PERGs allow us to derive the RCT as the difference between the VEP P100 and the PERG P50 peak latencies.[13,31]

Statistics

The differences between GP and GC patients and GC1 and GC2 patients and the differences observed in each group (GP, GC, GC1, and GC2) with respect to the baseline condition and to the examination previously performed were evaluated by one-way analysis of variance for repeated measures (ANOVA), and a P value < 0.01 was considered significant.

Results

Examples of simultaneous recordings of VEP and PERG before and after the medical treatment with citicoline or placebo are displayed in Figure 1. The mean data and the statistical analysis are shown in Table 1 and Figures 2–6.

At baseline, similar values for VEP and PERG parameters ($P >$ 0.05) in GC and GP patients were observed (Figs 2–6 "basal").

Visual Evoked Potential Recordings

First Period of Evaluation. In GP patients at 60 and 180 days, no significant changes ($P > 0.01$) of VEP parameters were observed with respect to the values observed at baseline ($P > 0.01$).

In GC patients at day 60, a decrease in P100 peak latencies and an increase in N75-P100 peak amplitudes ($P < 0.01$) with respect to the baseline values were found. The GC patients displayed P100 peak latencies shorter and N75-P100 peak amplitudes greater than those of GP patients ($P < 0.01$).

At day 180, an increase in P100 peak latencies and a decrease in N75-P100 peak amplitudes with respect to the values observed at 60 days were found. The VEP parameters observed were still shorter (P100 peak latency) and still greater (N75-P100 amplitude) than those observed at baseline ($P < 0.01$) and than those observed in GP patients ($P < 0.01$).

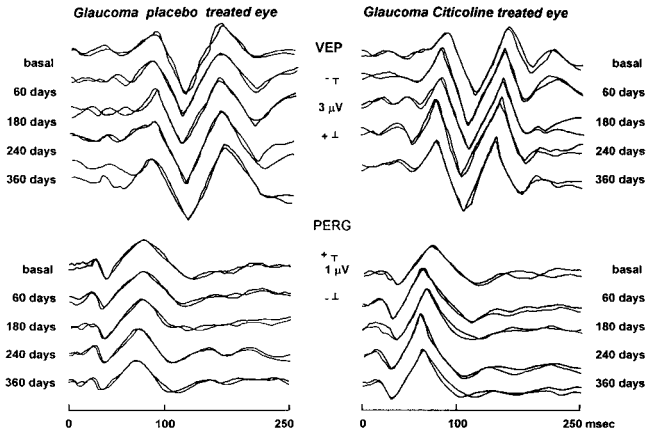

Figure 1. Layout of simultaneous VEP and PERG recordings in a patient with glaucoma treated with placebo (GP) and in a patient with glaucoma treated with citicoline (GC). Electrophysiologic examinations were assessed at baseline and at 60, 180, 240, and 360 days after medical treatment with placebo or citicoline. The treatment with placebo was performed in one 60-day period (0–60 days), followed by 300 days of washout. The citicoline treatment was performed in two different 60-day periods (0–60 and 181–240 days), followed by two periods of washout (61–180 and 241–360 days). In comparison with the baseline condition and GP patient, in the GC patient the PERG and VEP recorded after the citicoline treatment showed a decrease in peak latencies and an increase in amplitude, whereas in the GP patient the PERG and VEP layouts were similar to the baseline values.

Second Period of Evaluation. In GP patients at 240 and 360 days, unmodified electrophysiologic values were observed ($P >$ 0.01).

At day 300, GC1 patients presented a further increase in P100 peak latencies and a further decrease in N75-P100 peak amplitudes with respect to the values observed at day 180, and no differences between VEP parameters with respect to baseline and with respect to those of GP patients were found ($P > 0.01$).

At day 240 in GC2 patients, a further decrease in P100 peak latencies and a further increase in N75-P100 peak amplitudes ($P <$ 0.01) with respect to the values observed at day 180 day were observed. P100 peak latencies were still shorter and N75-P100 peak amplitudes were still greater than those of GP patients ($P <$ 0.01). At day 360, GC2 patients showed an increase in P100 peak latencies and a decrease in N75-P100 peak amplitudes with respect to the values observed at day 240. The values of P100 peak latencies and N75-P100 peak amplitudes were respectively still shorter and still greater than baseline values ($P < 0.01$) and than those of GP patients ($P < 0.01$).

Pattern-electretinogram Recordings

First Period of Evaluation. In GP patients at 60 and 180 days, unmodified PERG parameters were observed with respect to those observed at baseline ($P > 0.01$).

In GC patients at day 60, a decrease in P50 peak latencies and an increase in P50-N95 peak amplitudes ($P < 0.01$) with respect to the baseline values were found. The GC patients showed P50 peak latencies shorter and P50-N95 peak amplitudes greater than those of GP patients ($P < 0.01$).

After 120 days of washout (day 180), GC patients showed an increase in P50 peak latencies and a decrease in P50-N95 peak amplitudes with respect to the values observed at 60 days. Nev-

Table 1. Electrophysiologic Parameters Observed in Glaucoma Patients in Basal Condition and after Treatment with Citicoline (GC, GC1, and GC2 groups): ANOVA (A) versus Basal Condition, Examination Previously Performed and Glaucoma Patients Treated with Placebo (GP Group)

Group	Time	Mean ± SD	A versus Basal	A versus Previous	A versus GP Group (n = 15)
VEP P100 latency					
GC	basal	139.2 ± 9.30			f(1,38):0.03,*P*=0.860
(n = 25)	60 days (treatment)	125.7 ± 8.15	f(1,48):29.8,*P*=0.000	f(1,48):29.8,*P*=0.000	f(1,38):32.9,*P*=0.000
	180 days (follow-up)	130.6 ± 10.2	f(1,48):9.70,*P*=0.003	f(1,48):3.52,*P*=0.067	f(1,38):12.3,*P*=0.001
GC2	basal	139.4 ± 6.89			f(1,28):0.07,*P*=0.790
(n = 15)	180 days (follow-up)	131.6 ± 8.02	f(1,28):8.16,*P*=0.008		f(1,28):11.1,*P*=0.002
	240 days (treatment)	122.7 ± 8.21	f(1,28):36.4,*P*=0.000	f(1,28):9.02,*P*=0.006	f(1,28):36.4,*P*=0.000
	360 days (follow-up)	128.2 ± 6.70	f(1,28):20.3,*P*=0.000	f(1,28):4.04,*P*=0.054	f(1,28):19.1,*P*=0.000
GC1	basal	138.6 ± 5.95			f(1,23):0.01,*P*=0.972
(n = 10)	180 days (follow-up)	129.6 ± 6.64	f(1,18):11.4,*P*=0.003		f(1,23):16.1,*P*=0.000
	300 days (follow-up)	137.9 ± 6.32	f(1,18):0.07,*P*=0.802	f(1,18):8.19,*P*=0.010	f(1,23):0.52,*P*=0.478
VEP N75-P100 amplitude					
GC	basal	5.2 ± 2.70			f(1,38):6.23,*P*=0.636
(n = 25)	60 days (treatment)	8.1 ± 2.45	f(1,48):15.8,*P*=0.000	f(1,48):15.8,*P*=0.000	f(1,38):14.2,*P*=0.000
	180 days (follow-up)	7.1 ± 2.10	f(1,48):7.71,*P*=0.008	f(1,48):2.40,*P*=0.128	f(1,38):7.29,*P*=0.010
GC2	basal	5.12 ± 2.01			f(1,28):0.37,*P*=0.550
(n = 15)	180 days (follow-up)	7.0 ± 1.67	f(1,28):7.76,*P*=0.009		f(1,28):6.65,*P*=0.011
	240 days (treatment)	8.3 ± 1.39	f(1,28):25.3,*P*=0.000	f(1,28):5.37,*P*=0.028	f(1,28):19.6,*P*=0.000
	360 days (follow-up)	7.2 ± 1.20	f(1,28):9.87,*P*=0.004	f(1,28):4.06,*P*=0.053	f(1,28):8.26,*P*=0.008
GC1	basal	5.2 ± 1.93			f(1,23):0.20,*P*=0.657
(n = 10)	180 days (follow-up)	7.3 ± 1.01	f(1,18):9.74,*P*=0.006		f(1,23):8.29,*P*=0.007
	300 days (follow-up)	5.4 ± 1.58	f(1,18):0.06,*P*=0.803	f(1,18):10.2,*P*=0.005	f(1,23):0.18,*P*=0.679
PERG P50 latency					
GC	basal	69.3 ± 2.85			f(1,38):1.07,*P*=0.308
(n = 25)	60 days (treatment)	62.3 ± 3.10	f(1,48):69.1,*P*=0.000	f(1,48):68.1,*P*=0.000	f(1,38):72.6,*P*=0.000
	180 days (follow-up)	64.2 ± 3.60	f(1,48):30.8,*P*=0.000	f(1,48):4.00,*P*=0.051	f(1,38):38.6,*P*=0.000
GC2	basal	69.2 ± 2.40			f(1,28):1.34,*P*=0.256
(n = 15)	180 days (follow-up)	64.0 ± 2.75	f(1,28):30.4,*P*=0.000		f(1,28):47.0,*P*=0.000
	240 days (treatment)	61.2 ± 1.70	f(1,28):110, *P*=0.000	f(1,28):11.2,*P*=0.002	f(1,28):146, *P*=0.000
	360 days (follow-up)	62.9 ± 2.90	f(1,28):41.9,*P*=0.000	f(1,28):3.82,*P*=0.061	f(1,28):53.4,*P*=0.000
GC1	basal	69.4 ± 1.64			f(1,23):0.88,*P*=0.357
(n = 10)	180 days (follow-up)	64.3 ± 2.40	f(1,18):30.6,*P*=0.000		f(1,23):38.8,*P*=0.000
	300 days (follow-up)	68.2 ± 2.28	f(1,18):1.83,*P*=0.193	f(1,18):13.8,*P*=0.002	f(1,23):4.47,*P*=0.046
PERG P50-N95 amplitude					
GC	basal	0.69 ± 0.35			f(1,38):0.03,*P*=0.856
(n = 25)	60 days (treatment)	1.02 ± 0.40	f(1,48):9.64,*P*=0.003	f(1,48):9.64,*P*=0.003	f(1.38):8.79,*P*=0.005
	180 days (follow-up)	0.83 ± 0.45	f(1,48):1.51,*P*=0.225	f(1,48):2.49,*P*=0.121	f(1,38):1.26,*P*=0.268
GC2	basal	0.70 ± 0.31			f(1,28):0.01,*P*=0.930
(n = 15)	180 days (follow-up)	0.80 ± 0.31	f(1,28):0.78,*P*=0.384		f(1,28):1.07,*P*=0.310
	240 days (treatment)	1.12 ± 0.27	f(1,28):15.6,*P*=0.000	f(1,28):9.06,*P*=0.005	f(1,28):17.2,*P*=0.000
	360 days (follow-up)	0.92 ± 0.31	f(1,28):3.33,*P*=0.076	f(1,28):3.54,*P*=0.070	f(1,28):3.23,*P*=0.083
GC1	basal	0.68 ± 0.25			f(1,23):0.06,*P*=0.801
(n = 10)	180 days (follow-up)	0.87 ± 0.22	f(1,18):3.19,*P*=0.091		f(1,23):2.59,*P*=0.121
	300 days (follow-up)	0.71 ± 0.25	f(1,18):0.07,*P*=0.794	f(1,18):2.27,*P*=0.150	f(1,23):0.02,*P*=0.887
RCT (VEP 100 − PERG P50 latencies)					
GC	basal	70.7 ± 6.20			f(1,38):0.05,*P*=0.823
(n = 25)	60 days (treatment)	63.7 ± 6.21	f(1,48):13.4,*P*=0.000	f(1,48):13.4,*P*=0.000	f(1,38):10.6,*P*=0.002
	180 days (follow-up)	66.3 ± 6.22	f(1,48):5.04,*P*=0.029	f(1,48):2.06,*P*=0.158	f(1,38):7.06,*P*=0.010
GC2	basal	70.6 ± 6.02			f(1,28):0.03,*P*=0.856
(n = 15)	180 days (follow-up)	66.6 ± 5.81	f(1,28):3.44,*P*=0.074		f(1,28):5.34,*P*=0.028
	240 days (treatment)	62.4 ± 4.69	f(1,28):17.4,*P*=0.000	f(1,28):4.75,*P*=0.038	f(1,28):21.2,*P*=0.000
	360 days (follow-up)	65.8 ± 3.49	f(1,28):8.42,*P*=0.007	f(1,28):3.96,*P*=0.057	f(1,28):8.65,*P*=0.006
GC1	basal	70.8 ± 5.12			f(1,23):0.07,*P*=0.797
(n = 10)	180 days (follow-up)	66.0 ± 4.11	f(1,18):5.34,*P*=0.033		f(1,23):7.29,*P*=0.013
	300 days (follow-up)	70.0 ± 3.79	f(1,18):0.16,*P*=0.696	f(1,18):5.11,*P*=0.036	f(1,23):0.13,*P*=0.719

ertheless, the P50 peak latencies were still shorter than the baseline ones ($P < 0.01$) and than those of GP patients ($P < 0.01$), whereas the P50-N95 peak amplitudes were similar to those observed at baseline and similar to the GP values ($P > 0.01$).

Second Period of Evaluation. In GP patients at 240 and 360 days, unmodified electrophysiologic values were observed ($P > 0.01$).

In GC1 patients after a second period of 120 days of washout (day 300), a further increase in P50 peak latencies and a further decrease in P50-N95 peak amplitudes with respect to the values observed at 180 days were observed. The PERG parameters were similar to those of GP and were not significantly modified with respect to the baseline values ($P > 0.01$).

In GC2 patients after the second period of 60 days of citicoline treatment (day 240), a further decrease in P50 peak latencies and a further increase in P50-N95 peak amplitudes ($P < 0.01$) with

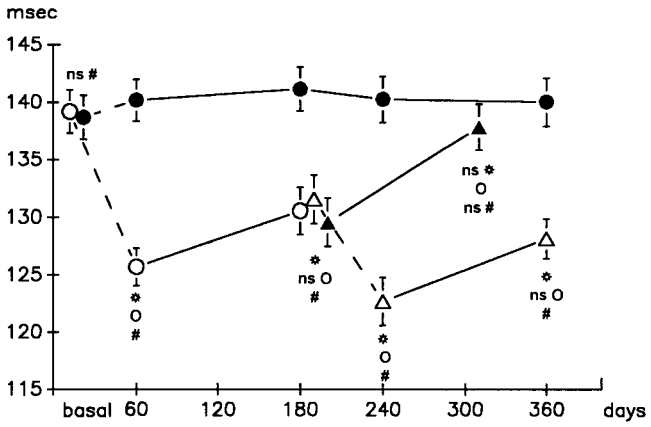

Figure 2. Mean values of VEP P100 peak latency observed in glaucoma patients at baseline and after medical treatment with placebo (GP, ●) or citicoline (GC, ○). The medical treatment with placebo or citicoline was performed over a 60-day period (0–60 days, dashed lines) followed by 120 days of washout (solid lines, 61–180 days). At day 180 the GC patients were divided into two groups: GC1 (▲), in which the washout was extended for another 120 days (solid lines, 181–300 days), and GC2 (△), in which a second 60-day period of citicoline treatment (dashed lines, 181–240 days) was followed by a second period of 180 days of washout (solid lines, 241–360 days). In GP patients, the period of washout was extended for another 180 days and VEPs were also recorded at 240 and 380 days. Vertical lines represent one standard error of the mean. Statistics: vs. baseline, *: $P < 0.01$; ns *: $P > 0.01$; vs. examination previously performed, ○: $P < 0.01$; ns ○ $P > 0.01$; vs. GP: #: $P < 0.01$, ns #: $P > 0.01$, (ANOVA).

respect to the values observed at 180 days were found. P50 peak latencies were still shorter and P50-N95 peak amplitudes were still greater than those of GP patients ($P < 0.01$).

At day 360, after a second period of 120 days of washout, GC2 patients displayed an increase in P50 peak latencies and a decrease in P50-N95 peak amplitudes with respect to the values observed at 240 days. Nevertheless, the P50 peak latencies were still shorter than the baseline ones ($P < 0.01$) and than those of GP patients ($P < 0.01$), whereas the P50-N95 peak amplitudes were similar to those observed in the baseline condition but significantly different from the GP ones ($P > 0.01$).

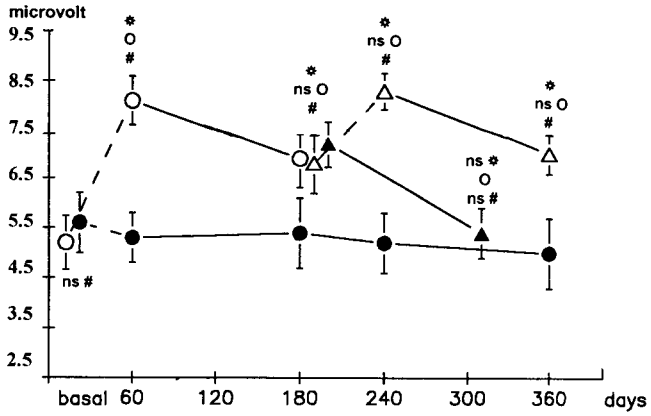

Figure 3. Mean values of VEP N75-P100 peak amplitude observed in glaucoma patients treated with placebo (●) or citicoline (GC: ○; GC1: ▲; GC2: △). Vertical lines represent one standard error of the mean. Statistics: refer to Figure 2.

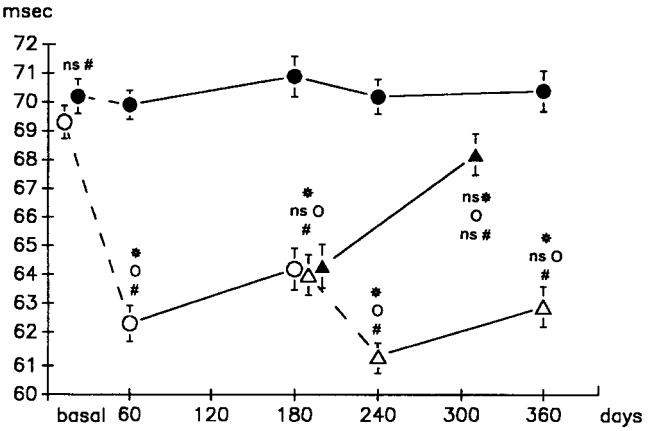

Figure 4. Mean values of PERG P50 peak latency observed in glaucoma patients treated with placebo (●) or citicoline (GC: ○; GC1: ▲; GC2: △). Vertical lines represent one standard error of the mean. Statistics: refer to Figure 2.

Retinocortical Time

First Period of Evaluation. In GP patients after 60 and 180 days, no changes in RCT were observed ($P > 0.01$).

In GC patients, at day 60 a decrease of RCT with respect to the baseline values was found ($P < 0.01$). At day 180, we observed an increase in RCT with respect to the values observed at day 60, and its values were not significantly modified with respect to baseline ones ($P > 0.01$).

Second Period of Evaluation. In GP patients at 240 and 360 days, unmodified ($P > 0.01$) RCT values were observed.

In GC1 patients at day 300, a further increase in RCT with respect to the values observed at 180 days was observed, and no differences ($P > 0.01$) with respect to the baseline values were found.

In GC2 patients at day 240, a decrease in RCT ($P < 0.01$) with respect to the values observed at 180 days was found. At day 360, an increase in RCT with respect to the values observed at 240 days was observed; nevertheless, the RCT values were still shorter than the baseline ones ($P < 0.01$).

In GC patients at days 60 and 180 and in GC2 patients at day 240, the RCT values were reduced with respect to those of GP patients ($P < 0.01$); at day 300, similar values between GC1 and

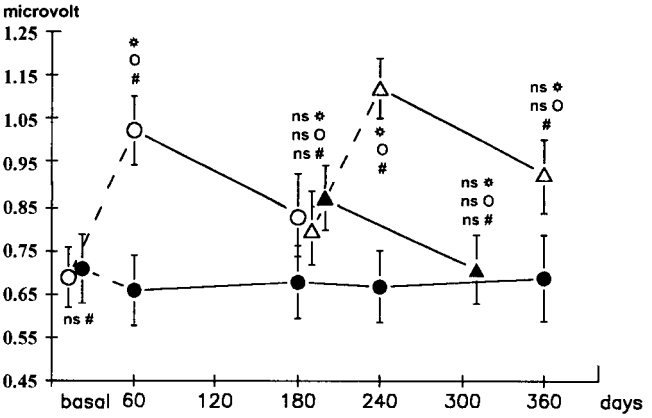

Figure 5. Mean values of PERG P50-N95 peak amplitude observed in glaucoma patients treated with placebo (●) or citicoline (GC: ○; GC1: ▲; GC2: △). Vertical lines represent one standard error of the mean. Statistics: refer to Figure 2.

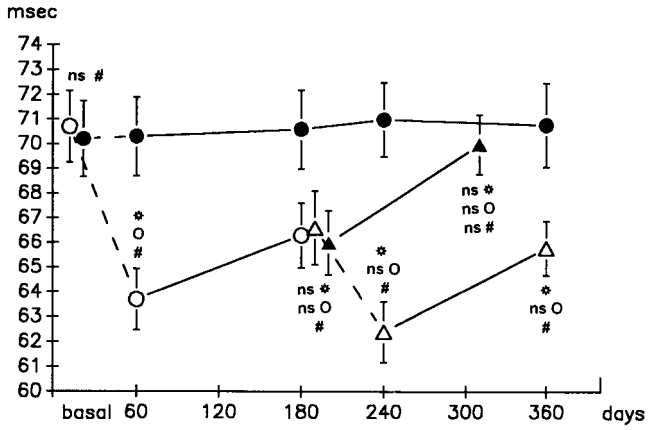

Figure 6. Mean values of RCT observed in glaucoma patients after medical treatment with placebo (●) or citicoline (GC: ○; GC1: ▲; GC2: △). Vertical lines represent one standard error of the mean. Statistics: refer to Figure 2.

GP patients were found ($P > 0.01$); at day 360, GC2 patients displayed RCT values still reduced with respect to those of GP patients ($P < 0.01$).

During the whole period of treatment, no adverse side effects were reported by any of the patients enrolled in the study. No significant changes in IOP were found in any of the subjects tested.

Discussion

The present study was designed to evaluate the retinal and visual cortical responses in patients with glaucoma treated with citicoline using simultaneous recordings of VEPs and PERGs.

We observed an improvement of cortical responses (VEP) in our glaucoma patients after treatment with citicoline, together with an improvement of retinal responses (PERG) and an improvement of the index of neural conduction in the postretinal visual pathways (RCT).

Although our results clearly show the effects of citicoline on the retinal and postretinal glaucomatous function, the mechanism of action of citicoline on visual function is not entirely understood and speculating on this is more difficult.

Citicoline is an endogenous substance that represents an obligatory intermediary for the synthesis of phosphatidylcholine, and it is a major phospholipid in the neuronal membrane.[34–37] It is also reported that citicoline, by activating the biosynthesis of structural phospholipids in the neuronal membranes, not only increases the metabolism of cerebral structures[36] but also inhibits phospholipid degradation.[37] A neuroprotective effect of citicoline has been suggested in situations of hypoxia and ischemia.[36,37] It has been proposed[36–41] that citicoline has a neuromodulator wide-action spectrum availability increasing the level in the central nervous system of different neurotransmitters and neuromodulators, including noradrenaline and dopamine. In several works, it has been observed that citicoline successfully increases the consciousness level in several brain disorders ascribed to vascular, traumatic, or degenerative processes.[42–47]

The definition of open-angle glaucoma includes an imbalance between blood pressure and ocular tension[48] that may result in ischemic damage inducing an involvement of the retinal[49–55] and postretinal[56,57] structures.

The above-mentioned vascular pathogenesis of glaucoma leads us to believe that such parallels with those diseases in which the therapeutic effects of citicoline were observed[42–47] can be drawn.

A possible increase in the consciousness level could explain the improved psychophysical responses evaluated by visual field analysis observed in glaucoma patients after treatment with citicoline.[7] To explain the improvement of VEP and PERG responses, other effects of citicoline (apart from the increased consciousness level) must be evaluated in those visual structures that are known to be involved in glaucoma and, in particular, at the retinal and postretinal levels.

That citicoline has effects on the visual system has recently been suggested by the improvement of visual acuity,[35,58] VEP responses, and contrast sensitivity[59] in amblyopic subjects after treatment with this substance. In this latter study, a dopaminergic-like activity has been suggested by similar results obtained in amblyopic subjects after treatment with levodopa[60–62] or citicoline[58,59] and from studies performed in patients with Parkinson disease in whom citicoline was used as a complement to levodopa therapy.[63–65]

As a result of this property it is possible to explain, at least in part, the effects observed in our study. Visual evoked responses were improved by citicoline in patients with glaucoma, and these electrophysiologic data showing that citicoline improves the cortical responses confirm all that was previously suggested by psychophysical analysis.[7]

In the VEP improvement, a dopaminergic-like activity of citicoline could be proposed; in fact, it is known that levodopa induces a shortening of VEP latency in humans, with a possible retinal contribution considering that the PERG latencies are also shortened by this substance.[66]

Because VEP abnormalities have recently been ascribed to a dysfunction in the innermost retinal layers (ganglion cells and their fibers) related to an impaired neural conduction in the postretinal visual pathways,[30] we also evaluated the effects of citicoline on retinal function and on neural conduction in the visual pathways.

Retinal function was assessed by PERG recordings, and after treatment with citicoline we found an improvement of PERG parameters. Given that in glaucoma a loss of ganglion cells and their fibers has been documented by histologic studies[49–52] and by objective methods of morphologic evaluation in vivo of the retinal fibers,[53–55] the impaired PERG responses observed in patients with glaucoma could be ascribed to a dysfunction of the innermost retinal layers, although a functional impairment of the preganglionic elements has also been suggested.[67,68]

Our results indicate that citicoline improves bioelectric retinal activity, but we are not able to demonstrate whether there were other effects on the retinal fibers (i.e., an increase in retinal nerve fiber layer thickness) because we have evaluated only the retinal function and have not performed any morphologic examination.

The PERG improvement could also be ascribed to a dopaminergic-like activity of citicoline; in fact, levodopa was found to increase the retinal function in humans treated with this substance.[66] Therefore, our results could be explained by a similar neuromodulator activity.

Further to the retinal function assessment, we evaluated the neural conduction in the visual pathways by measurements of RCT. It is unlikely that RCT represents the real transit time of neural conduction between the retina and the visual cortex (we do not believe that bioelectric signals take 50 ms to travel from the retina to the visual cortex in normal subjects, as we previously observed[30,31] and as was previously reported by Celesia et al[14]), but it could be considered as an "index" of neural conduction in the postretinal visual pathways.

After treatment with citicoline, a reduced RCT was observed in our glaucoma patients. The reduced RCT could be ascribed to an improvement in the retinal function with consequent better neural conduction in the visual pathways and relative increased bioelectric activity in those cells in which the cortical potentials have their source. All of this could also explain the reduced VEP P100 latencies and the increased VEP N75-P100 amplitudes observed after treatment with citicoline. An independent effect of citicoline on neural conduction in the postretinal visual pathways or in the visual cortical cells could be supposed, but for this hypothesis we have no clear or conclusive appropriate experimental or written data.

As we have recently observed that perimetrical indexes (mean deviation of Humphrey perimetry) are significantly related to PERG and VEP parameters and to RCT,[69] it is likely that the above-mentioned sources of cortical improvement could also be suggested as an explanation for the better perimetric condition observed after treatment with citicoline.[7]

In our study we assessed the long-term effects of citicoline. In glaucoma patients in which only one period of treatment was performed (GC1 group), after 120 days of washout we observed that the therapeutic effects were still present, whereas after 240 days of washout all electrophysiologic parameters were similar to those observed before the start of the treatment. All this suggests that retinal and cortical responses are still improved at 120 days from the end of the treatment, while it is not possible to observe any therapeutic effect on retinal and visual pathway functions after 240 days of washout.

In conclusion, citicoline is significant in improving the retinal and cortical responses in glaucoma patients. This effect cannot be considered specific for glaucoma patients. In fact, an improvement of VEP responses has been previously observed after treatment with citicoline in normal as well as in amblyopic eyes.[59]

Our results, together with those previously observed by visual field analysis,[7] indicate a potential use for this substance in the medical treatment of glaucoma as a complement to hypotensive therapy. Toward this end and in agreement with previous observations in similar studies,[35–37,58,59] a positive step is represented by the lack of adverse pharmacologic side effects reported from any of the subjects participating in this study.

Acknowledgments. We thank Dr. Vittorio Porciatti for helpful discussion and critical reading of the manuscript. The authors also thank Nuovo Consorzio Sanitario Nazionale, Rome, Italy, for kindly providing Neuroton and placebo.

References

1. Krieglstein GK, Schrems W, Gramer E, Leydhecker W. Detectability of early glaucomatous field defects. A controlled comparison of Goldmann versus Octopus perimetry. In: Greve EL, Verriest G, eds. Fourth International Visual Field Symposium, 1980. The Hague: Dr W Junk, 1981; 19–24 (Doc Ophthalmol Proc Ser; 26).
2. Werner EB, Piltz-Seymour J. What constitutes a glaucomatous visual field defect. Seminars in Ophthalmology 1992;7:110–9.
3. Gunduz K, Arden GB, Perry S, et al. Color vision defects in ocular hypertension and glaucoma. Quantification with a computer-driven color television system. Arch Ophthalmol 1988; 106:929–35.
4. Arden GB, Jacobson JJ. A simple grating test of contrast sensitivity: preliminary results indicate value in screening for glaucoma. Invest Ophthalmol Vis Sci 1978;17:23–32.
5. Hitchings RA, Powell DJ, Arden GB, Carter RM. Contrast sensitivity gratings in glaucoma family screening. Br J Ophthalmol 1981;65:515–7.
6. Pomerance GN, Evans DW. Test-retest reliability of the CVS-1000 contrast test and its relationship to glaucoma therapy. Invest Ophthalmol Vis Sci 1994;35:3357–61.
7. Pecori Giraldi J, Virno M, Covelli G, et al. Therapeutic value of citicoline in the treatment of glaucoma (computerized and automated perimetric investigation). Int Ophthalmol 1989;13: 109–12.
8. Maffei L, Fiorentini A. Electroretinographic responses to alternating gratings before and after section of the optic nerve. Science 1981;211:953–5.
9. Maffei L, Fiorentini A. Electroretinographic responses to alternating gratings in the cat. Exp Brain Res 1982;48:327–34.
10. Hollander H, Bisti S, Maffei L, Hebel R. Electroretinographic responses and retrograde changes of retinal morphology after intracranial optic nerve section. A quantitative analysis in the cat. Exp Brain Res 1984;55:483–93.
11. Maffei L, Fiorentini A, Bisti S, Hollander H. Pattern ERG in the monkey after section of the optic nerve. Exp Brain Res 1985;59:423–5.
12. Trimarchi C, Biral G, Domenici L, et al. The flash- and pattern-electroretinogram generators in the cat: a pharmacological approach. Clinical Vision Sciences 1990;6:19–24.
13. Celesia GG, Kaufman D. Pattern ERGs and visual evoked potentials in maculopathies and optic nerve diseases. Invest Ophthalmol Vis Sci 1985;26:726–35.
14. Celesia GG, Kaufman D, Cone SB. Simultaneous recording of pattern electroretinography and visual evoked potentials in multiple sclerosis. A method to separate demyelination from axonal damage to the optic nerve. Arch Neurol 1986;43:1247–52.
15. Siliprandi R, Bucci MG, Canella R, Carmignoto G. Flash and pattern electroretinograms during and after acute intraocular pressure elevation in cat. Invest Ophthalmol Vis Sci 1988;29: 558–65.
16. Marx MS, Podos SM, Bodis-Wollner I, et al. Flash and pattern electroretinograms in normal and laser-induced glaucomatous primate eyes. Invest Ophthalmol Vis Sci 1986;27:378–86.
17. Graham SL, Wong VAT, Drance SM, Mikelberg FS. Pattern electroretinograms from hemifields in normal subjects and

patients with glaucoma. Invest Ophthalmol Vis Sci 1994;35: 3347–56.
18. Johnson MA, Drum BA, Quigley HA, et al. Pattern-evoked potentials and optic nerve fiber loss in monocular laser-induced glaucoma. Invest Ophthalmol Vis Sci 1989;30:897–907.
19. Marx MS, Bodis-Wollner I, Lustgarten JS, Podos SM. Electrophysiological evidence that early glaucoma affects foveal vision. Doc Ophthalmol 1987;67:281–301.
20. Marx MS, Bodis-Wollner I, Podos SM, Teitelbaun CS. The pattern ERG and VEP in glaucomatous optic nerve disease in the monkey and human. In: Cracco RQ, Bodis-Wollner I, eds. Evoked Potentials. New York: Liss, 1986; 117–26 (Frontiers of Clinical Neuroscience; V. 3).
21. O'Donaghue E, Arden GB, O'Sullivan F, et al. The pattern electroretinogram in glaucoma and ocular hypertension. Br J Ophthalmol 1992;76:387–94.
22. Pfeiffer N, Tillmon B, Bach M. Predictive value of the pattern electroretinogram in high-risk ocular hypertension. Invest Ophthalmol Vis Sci 1993;34:1710–5.
23. Porciatti V, Falsini B, Brunori S, et al. Pattern electroretinogram as a function of spatial frequency in ocular hypertension and early glaucoma. Doc Ophthalmol 1987;65:349–55.
24. Wanger P, Persson HE. Pattern-reversal electroretinograms in unilateral glaucoma. Invest Ophthalmol Vis Sci 1983;24:749–53.
25. Watanabe I, Iijima H, Tsukahara S. The pattern electroretinogram in glaucoma: an evaluation by relative amplitude from the Bjerrum area. Br J Ophthalmol 1989;73:131–5.
26. Bray LC, Mitchell KW, Howe JW. Prognostic significance of the pattern visual evoked potential in ocular hypertension. Br J Ophthalmol 1991;75:79–83.
27. Bucci MG, Parisi V, Giannini R, Rossini PM. Recordings of visual evoked potentials after photostress in artificially increased intraocular pressure. Clinical Vision Sciences 1991;6: 431–6.
28. Parisi V, Bucci MG. Visual evoked potentials after photostress in patients with primary open-angle glaucoma and ocular hypertension. Invest Ophthalmol Vis Sci 1992;33:436–42.
29. Vaegan, Graham SL, Goldberg I, et al. Flash and pattern electroretinogram changes with optic atrophy and glaucoma. Exp Eye Res 1995;60:697–706.
30. Parisi V. Neural conduction in the visual pathways in ocular hypertension and glaucoma. Graefes Arch Clin Exp Ophthalmol 1997;235:136–42.
31. Parisi V, Uccioli L, Parisi L, et al. Neural conduction in visual pathways in newly-diagnosed IDDM patients. Electroencephalogr Clin Neurophysiol 1998;108:490–6.
32. Jasper HH. The ten-twenty electrode system of the International Federation of Electroencephalography. Clin Neurophysiol 1958;10:371–5.
33. Fiorentini A, Maffei L, Pirchio M, et al. The ERG in response to alternating gratings in patients with diseases of the peripheral visual pathway. Invest Ophthalmol Vis Sci 1981; 21: 490–3.
34. Goracci G, Francescangeli E, Mozzi R, et al. Regulation of phospholipid metabolism by nucleotides in brain and transport of CDP-choline into brain. In: Novel Biochemical, Pharmacological and Clinical Aspects of Cytidinediphosphocholine: Proceedings of the International Meeting, 1984: Sorrento, Italy. New York: Elsevier, 1985; 105–9.
35. Kennedy EP. Biosynthesis of phospholipids. Fed Proc Balt 1957;16:847–53.
36. Secades JJ, Frontera G. CDP-choline: pharmacological and clinical review. Methods Find Exp Clin Pharmacol 1995; 17(Suppl B):2–54.
37. Weiss GB. Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. Life Sci 1995;56:637–60.
38. Campos EC. Future directions in the treatment of amblyopia. Lancet 1997;349:1190.
39. Canonico PL, Nicoletti F, Sortino MA, et al. Effects of CDP-choline administration on receptor-stimulated inositol phospholipid hydrolysis in brain slices and neuronal cultures. Funct Neurol 1992;7:275–81.
40. Fonlupt P, Martinet M, Pacheco H. Effect of CDP-choline on dopamine metabolism in the central nervous system. In: Novel Biochemical, Pharmacological and Clinical Aspects of Cytidinediphosphocholine: Proceedings of the International Meeting, 1984: Sorrento, Italy. New York: Elsevier, 1985; 169–77.
41. Martinet M, Fonlupt P, Pacheco H. Effects of cytidine-5′diphosphocholine on norepinephrine, dopamine and serotonin synthesis in various regions of the rat brain. Arch Int Pharmacodyn Ther 1979;239: 52–61.
42. Agnoli A, Fioravanti M, Lechner H. Efficacy of CDP-choline in chronic cerebral vascular diseases (CCVD). In: Novel Biochemical, Pharmacological and Clinical Aspects of Cytidinediphosphocholine: Proceedings of the International Meeting, 1984: Sorrento, Italy. New York: Elsevier, 1985;305–8.
43. Boismare F, Le Poncin M, Lefrancois J, Lecordier JC. Action of cytidine diphosphocholine on functional and hemodynamic effects of cerebral ischemia in cats. Pharmacology 1978;17: 15–20.
44. Cacabelos R, Caamano J, Gomez MJ, et al. Therapeutic effects of CDP-choline in Alzheimer's disease. Cognition, brain mapping, cerebrovascular hemodynamics, and immune factors. Ann N Y Acad Sci 1996;777:399–403.
45. Kakihana M, Fukuda N, Suno M, Nagaoka A. Effects of CDP-choline on neurologic deficits and cerebral glucose metabolism in a rat model of cerebral ischemia. Stroke 1988;19: 217–22.
46. Serra I, Alberghina M, Viola M, et al. Effect of CDP-choline on the biosynthesis of nucleic acids and proteins in brain regions during hypoxia. Neurochem Res 1981;6:607–18.
47. Zappia V, Kennedy P, Nilsson BI, Galletti P, eds. Novel Biochemical, Pharmacological and Clinical Aspects of Cytidinediphosphocholine. New York: Elsevier; 1985.
48. Drance SM. The vascular factors in glaucoma. In: Bucci MG, ed. Glaucoma: Decision Making in Therapy. Berlin; New York: Springer, 1996;31–5.
49. Quigley HA, Addicks EM, Green WR. Optic nerve damage in human glaucoma. III. Quantitative correlation of nerve fiber loss and visual field defect in glaucoma, ischemic neuropathy, papilledema, and toxic neuropathy. Arch Ophthalmol 1982; 100:135–46.
50. Quigley HA, Sanchez RM, Dunkelberger GR, et al. Chronic glaucoma selectively damages large optic nerve fibers. Invest Ophthalmol Vis Sci 1987;28:913–20.
51. Quigley HA, Dunkelberger GR, Green WR. Chronic human glaucoma causing selectively greater loss of large optic nerve fibers. Ophthalmology 1988;95:357–63.
52. Quigley HA, Nickells RW, Kerrigan LA, et al. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. Invest Ophthalmol Vis Sci 1995;36:774–86.
53. Orzalesi N, Miglior S, Lonati C, Rosetti L. Microperimetry of localized retinal nerve fiber layer defects. Vision Res 1998; 38:763–71.
54. Shuman JS, Hee MR, Puliafito CA, et al. Quantification of nerve fiber layer thickness in normal and glaucomatous eyes using optical coherence tomography. Arch Ophthalmol 1995; 113:586–96.

55. Yucel YH, Gupta N, Kalichman MW, et al. Relationship of optic disc topography to optic nerve fiber number in glaucoma. Arch Ophthalmol 1998;116:493–7.
56. Chaturvedi N, Hedley-Whyte ET, Dreyer EB. Lateral geniculate nucleus in glaucoma. Am J Ophthalmol 1993;116:182–8.
57. Dandona L, Hendrickson A, Quigley HA. Selective effects of experimental glaucoma on axonal transport by retinal ganglion cells to the dorsal lateral geniculate nucleus. Invest Ophthalmol Vis Sci 1991;32:1593–9.
58. Campos EC, Schiavi C, Benedetti P, et al. Effect of citicoline on visual acuity in amblyopia: preliminary results. Graefes Arch Clin Exp Ophthalmol 1995;233:307–12.
59. Porciatti V, Schiavi C, Benedetti P, et al. Cytidine-5′-diphosphocholine improves visual activity, contrast sensitivity and visually-evoked potentials of amblyopic subjects. Curr Eye Res 1998;17:141–8.
60. Gottlob I, Stangler-Zuschrott E. Effect of levodopa on contrast sensitivity and scotomas in human amblyopia. Invest Ophthalmol Vis Sci 1990;31:776–80.
61. Leguire LE, Rogers GL, Bremer DL, et al. Levodopa/carbidopa for childhood amblyopia. Invest Ophthalmol Vis Sci 1993;34:3090–5.
62. Gottlob I, Charlier J, Reinecke RD. Visual acuities and scotomas after one week levodopa administration in human amblyopia. Invest Ophthalmol Vis Sci 1992;33:2722–8.
63. Agnoli A, Ruggieri S, Denaro A, Bruno G. New strategies in the management of Parkinson's disease: a biological approach using a phospholipid precursor (CDP-choline). Neuropsychobiology 1982;8:289–96.
64. Birbamer G, Gesterbrand E, Rainer J, Eberhardt R. CDP-choline in the treatment of Parkinson's disease. New Trends in Clinical Pharmacology 1990;4:1–6.
65. Manaka S, Fuchinoue T, Sakino H, Sano K. Mechanism of action of CDP-choline in Parkinsonism. Brain and Nerve 1971;24:1051–66.
66. Gottlob I, Weghaupt H, Vass C, Auff E. Effect of levodopa on the human pattern electroretinogram and pattern visual evoked potentials. Graefes Arch Clin Exp Ophthalmol 1989;227:421–7.
67. Falsini B, Colotto A, Porciatti V, et al. Macular flicker- and pattern-ERGs are differently affected in ocular hypertension and glaucoma. Clinical Vision Sciences 1991;6:423–9.
68. Holopigian K, Seiple W, Mayron C, et al. Electrophysiological and psychophysical flicker sensitivity in patients with primary open-angle glaucoma and ocular hypertension. Invest Ophthalmol Vis Sci 1990;31:1863–8.
69. Parisi V, Manni GL, Sgrulletta R, et al. Delayed postretinal neural conduction in glaucoma patients: correlations between electrophysiological and computerized static perimetry parameters. Acta Ophthalmol Scand Suppl 1997;224:31–2.