Neuroprotection in Primary Open-Angle Glaucoma: The Role of a Fixed Citicoline-Homotaurine-Vitamin E Combination

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Abstract: Glaucomatous optic neuropathy is a chronic degenerative neuropathy characterized by progressive damage of the retinal ganglion cells despite good compensation of intraocular pressure. The purpose of this study was to assess the effect of oral administration of a fixed combination of citicoline 500 mg + homotaurine 50 mg + vitamin E 12 mg (CIT/HOMO) on retinal ganglion cell function as examined by pattern electroretinogram (PERG) in subjects with primary open-angle glaucoma. A prospective, randomized, single-blind, balanced, crossover study was performed on a population of 40 patients with POAG-HT and fully-compensated IOP with topical hypotensive therapy. Recruited patients were allocated by balancing randomization to two treatment groups: - group A: patients continued current hypotensive eye-drop for 4 months and subsequently took 1 tablet of CIT/HOMO each morning for 4 months; - group B: patients took 1 tablet of CIT/HOMO each morning for 4 months in addition to current hypotensive eye-drop and subsequently continued with current hypotensive eye-drop alone for 4 months. Patients were examined at baseline (T0), after 4 (T1) and 8 months (T2). At every single time was performed a whole eye examination, 3 IOP measurements, 30.2 SITA Standard Humphrey visual field test, OCT cup/disc ratio and PERG glaucoma Hemifield test with central amplitude analysis. 38 patients completed the study for a total of 76 eyes. In both groups of patients tonometry, cup/disc ratio and visual field did not reveal any statistically significant difference. In both groups, adding the CIT/HOMO at hypotensive eye-drop resulted in an improvement in PERG after 4 months of therapy that disappeared when CIT/HOMO was withdrawn. Four months supplementation with a fixed combination of citicoline, homotaurine and vitamin E was seen to significantly increase the amplitude of the PERG bioelectric potential transmitted by the optical pathway to the visual cortex in subjects with primary open-angle glaucoma with compensated IOP and initial damage of the visual field and optic disc. During this study, the IOP remained compensated with the current hypotensive therapy and no deterioration was observed in the visual field or the cup/disc ratio.

Keywords: Primary Open-angle Glaucoma, Citicoline, Homotaurine, Vitamin E, PERG

1. Introduction

Glaucomatous optic neuropathy is a chronic degenerative disease characterized by progressive damage to the retinal ganglion cells despite good IOP compensation.

The early stages of glaucomatous optic neuropathy are characterized by: blockage of axonal transport, excessive intracytoplasmic calcium levels, mitochondrial dysfunction, oxidative stress, and abnormal intra- and extracellular protein accumulation [1-3].

The same processes can be observed in pathological ageing of the brain, such as Alzheimer’s disease, and they result in the activation of ganglion cell apoptosis.

Counteracting intracellular oxidative processes or the extracellular accumulation of pathological matrices can have a neuroprotective effect [4-6].
Citocoline and Homotaurine are two compounds that have been extensively investigated in experimental models and clinical trials regarding progressive degenerative diseases of the central nervous system (CNS).

CDP-choline is an endogenous compound that is naturally found in cell membranes. It is a biosynthetic intermediate of the synthesis of phosphatidylcholine, the major phospholipid present in cell membranes. When administered exogenously it can be called citocoline. Orally administered citocoline is rapidly absorbed and hydrolyzed into choline and cytidine in the wall of the intestine. In the CNS, citocoline causes an increase in the synthesis of phospholipids of the neuronal and mitochondrial membranes, it reduces the release of free fatty acids and increases the levels of neurotransmitters such as dopamine (DA), serotonin (5-HT), sodium (NA) and acetylcholine by acting as a choline donor. It therefore helps to protect the neuronal structure from damage of various origins and improves membrane function [7]. Citocoline is a compound that has been extensively studied in neurodegenerative diseases of the CNS (stroke, cognitive impairment, sequelae of head injuries) and in subjects with glaucoma, where positive effects have been detected by visual field analysis, pattern electroretinography (PERG) and visual evoked potentials (VEP) [8-10].

Homotaurine (3-aminopropane sulphonate) is a small natural compound that is found in a number of red seaweeds that is first extracted from the algae and subsequently chemically synthesized and placed on the pharmaceutical market with the name tramiprosate [11]. Homotaurine has been seen to prevent the neurotoxicity induced by β-amyloid (Aβ) by reducing the aggregation of peptide fibrils and was the first disease-modifying fibrillogenesis inhibitor to be used in a phase 3 clinical trial on Alzheimer’s disease [12, 13]. The inhibition of Aβ deposition could support cholinergic transmission as the peptide has been seen to damage neurotransmission also by inhibiting choline-acetyltransferase (ChAT) (an enzyme that is indispensable for the biosynthesis of acetylcholine from colina+acetil-CoA), therefore potentially completing the cholinergic effect of citocoline [14]. Its mechanism of action is also associated with the affinity of the GABA-A receptor that allows a reduction in the apoptotic cascade mediated by Aβ oligomers, but also by conditions of ischemic stress or trophic deprivation.

The preclinical and clinical studies conducted with homotaurine regarding a number of neurodegenerative diseases have demonstrated that it has a significant effect on neuronal survival, with a reduction in the loss of volume of the hippocampus and critical areas of the brain, a decrease in infarct volume in ischemic stroke models and an improvement in the neurophysiological parameters of cholinergic transmission such as the short-latency afferent inhibition (SLAI). These analyses have been conducted on Alzheimer’s patients and subjects with mild cognitive impairment (MCI) [13, 15, 16].

One in vitro study showed that treatment with citocoline and homotaurine has a synergistic positive effect in retinal cell cultures under experimental conditions consistent with neuroretinal degeneration [17]. Vitamin E (tocopherol) has an important protective action on cell membranes, protecting them against oxidation by free radicals, it inhibits platelet aggregation and supports cell turnover [21]. The possible synergistic action of these substances administered as a fixed combination could play a role in limiting the apoptosis of the retinal ganglion cells of patients with hypertensive primary open-angle glaucoma (POAG-HT).

The purpose of this study was to assess the effect of oral administration of a fixed combination of citocoline 500 mg + homotaurine 50 mg + vitamin E 12 mg (CIT/HOMO) on retinal ganglion cell function examined by pattern electroretinography in subjects with primary open-angle glaucoma.

### 2. Materials and Methods

The study was conducted in accordance with the principles of the Declaration of Helsinki (Revision 2000) and the Italian Legislation on Good Clinical Practices (DM July 15 1997 and update December 16 2014). All eligible patients received detailed information on the characteristics of the nutraceutical CIT/HOMO to be taken and on all the procedures they would undergo, and gave their informed consent.

A prospective, randomised, single-blind, balanced, crossover study on a population of 40 patients with POAG-HT and fully-compensated IOP receiving topical hypotensive therapy.

All patients were examined by the same researcher (CC), who was blinded to the treatment assigned to the patients. The researcher who performed randomization (VL) and the other researchers had free access to all patient data.

The patients were recruited using the criteria indicated in table 1.

<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Exclusion</th>
</tr>
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<tbody>
<tr>
<td>age between 40 and 79 years</td>
<td>other eye conditions</td>
</tr>
<tr>
<td>diagnosis of bilateral POAG-HT for at least 1 year</td>
<td>prior eye surgery</td>
</tr>
<tr>
<td>topical hypotensive therapy other than brimonidine for at least 1 year</td>
<td>neurodegenerative disease or cerebrovascular accident or diabetes</td>
</tr>
<tr>
<td>mean IOP ≤ 19 in both eyes on 3 measurements (08:00-12:00-16:00)</td>
<td>arterial hypertension or hypotension</td>
</tr>
<tr>
<td>at least 3 parametric tests using a Humphrey 30.2 SITA Standard analyser: max 8 points with absolute defect, MD between -1 and -10, loss of fixation ≤ 20%, PF and NF ≤ 10%</td>
<td>sleep apnoea</td>
</tr>
<tr>
<td>Cup/disc ratio at OCT between 0.2 and 0.4</td>
<td>use of any type of dietary supplement or neuroprotectors in the 6 months prior to inclusion.</td>
</tr>
<tr>
<td>corneal pachymetry within the normal range corrected visual acuity not less than 7/10</td>
<td>participation in other clinical trials</td>
</tr>
<tr>
<td>refractive errors between 3D sph and 1D cyl</td>
<td>topical brimonidine therapy</td>
</tr>
</tbody>
</table>

### Table 1. Eligibility criteria.
In order to evaluate the influence of the nutraceutical CIT/HOMO on both eyes of the recruited patients, the following tests were conducted prior to randomization (T0) and at the end of each treatment period (T1 and T2): eye exam, 3 IOP measurements, visual field test using Humphrey 30.2 SITA Standard, OCT cup/disc ratio, PERG glaucoma hemifield test with central amplitude analysis.

Pattern electroretinography (PERG) consists in the presentation of a structured stimulus of varying magnitudes and light intensities that certainly involves the macula. The electrophysiological responses evoked by PERG is attributed to the inner layer of the retina and, in particular, the ganglion cells. Stimuli with high spatial frequencies evoke responses from the macular region, whereas stimuli with lower spatial frequencies evoke responses from the more peripheral regions. The stimulus can be transient (which consists in changes in the low-frequency structured stimulus between white and black, around 2 Hertz) or steady state (with changes in the high-frequency stimulus, 16 Hz).

In this study, potentials were detected using the transient stimulus. The evoked response is constituted by a small negative wave preceding a broad positive wave followed by a broad negative component, these peaks are labelled by a letter indicating the polarity of the peak and a number indicating the peak culmination time (N35, P50, N95). In this study, we considered the N35 - P50 and P50 - N95 peak-to-peak amplitude, as this is the component that undergoes the greatest changes in early-stage glaucoma [18-20].

The 40 recruited patients were allocated by balancing randomisation to two treatment groups (Figure 1):

1. Group A (20 patients): continued current IOP therapy for 4 months (T1) and subsequently also took 1 tablet of CIT/HOMO every morning for 4 months (T2).
2. Group B (20 patients): in addition to current IOP therapy patients took 1 tablet of CIT/HOMO each morning for 4 months (T1) and subsequently continued with current IOP therapy alone for 4 months (T2).

4. Results

We enrolled 40 subjects, of whom 27 females (67.5%) and 13 males (32.5%). The study was completed by 38 subjects, for a total of 76 eyes.

Two subjects dropped out of the study because of gastrointestinal disorders during use of the nutraceutical. At T0, no statistically significant differences were observed between patients allocated to Group A and those allocated to Group B (Table 2).

Table 2. Differences between patients allocated to the two treatment groups at baseline.

<table>
<thead>
<tr>
<th>T0=Baseline</th>
<th>Group A</th>
<th>Group B</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IOP (mmHg)*</td>
<td>16.00 (15.50 – 16.50)</td>
<td>16.00 (16.00 – 16.00)</td>
<td>0.39</td>
</tr>
<tr>
<td>Cup-to-disc*</td>
<td>0.30 (0.25 – 0.40)</td>
<td>0.30 (0.25 – 0.35)</td>
<td>0.84</td>
</tr>
<tr>
<td>N35-P50 (µV)**</td>
<td>5.70±2.08</td>
<td>5.20±2.11</td>
<td>0.25</td>
</tr>
<tr>
<td>P50-N95 (µV)**</td>
<td>5.51±2.00</td>
<td>5.20±2.09</td>
<td>0.50</td>
</tr>
<tr>
<td>MD (dB)*</td>
<td>-3.95 (-6.01 – -0.75)</td>
<td>-2.47 (-4.74 – -1.15)</td>
<td>0.42</td>
</tr>
</tbody>
</table>

The data are presented as mean standard deviation.

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During the study period, no significant differences were observed in IOP, cup/disc ratio and mean defect of the visual field compared to the baseline values.

Significant differences were observed for the PERG N35-P50 and P50-N95 amplitudes.

At T1 (4 months) the PERG N35-P50 and P50-N95 amplitude showed a statistically significant increase amongst subjects in Group B (PERG N35-P50 µV*: 6.66±2.09 vs 5.17±2.04, p 0.0001; PERG P50-N95 6.65±2.10 vs 5.20±2.09, p<0.0001).

At T2 (8 months) the PERG N35-P50 and P50-N95 amplitude underwent a significant increase amongst subjects in Group A (PERG N35-P50 µV*: 7.56±2.04 vs 5.50±2.11, p 0.0001; PERG P50-N95: 7.56±2.34 vs 5.45±2.01).

Figure 2 shows the N35-P50 amplitude trend at baseline, at
4 months and at 8 months in the two treatment groups:

Group A: IOP therapy alone for 4 months followed by IOP + CIT/HOMO for a further 4 months.

Group B: IOP therapy + CIT/HOMO for 4 months followed by IOP therapy alone for a further 4 months.

Group B: Hypotensive therapy + CIT/HOMO for 4 months followed by hypotensive therapy alone for a further 4 months.

The crossover analysis confirmed the absence of differences between the groups, with the exception of the PERG N35-P50 and P50-N95 amplitude.

Table 3 summarizes the crossover analysis. A significant difference was observed between the treatment (A vs B) in terms of the PERG values (p<0.0001), which demonstrates that treatment with CIT/HOMO improved the PERG amplitude during the period analyzed.

The absence of significance between AB vs BA indicates an absence of carryover.

Table 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>beta±SE</th>
<th>beta±SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N35-P50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs. B</td>
<td>5.41±0.25</td>
<td>7.17±0.25</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AB vs. BA</td>
<td>6.67±0.34</td>
<td>5.91±0.34</td>
<td>0.31</td>
</tr>
<tr>
<td>P50-N95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs. B</td>
<td>5.32±0.24</td>
<td>7.10±0.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>AB vs BA</td>
<td>6.52±0.34</td>
<td>5.90±0.34</td>
<td>0.19</td>
</tr>
<tr>
<td>Cup-to-disc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs. B</td>
<td>0.29±0.01</td>
<td>0.29±0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>AB vs. BA</td>
<td>0.30±0.01</td>
<td>0.29±0.01</td>
<td>0.69</td>
</tr>
<tr>
<td>MD of Visual Field</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A vs. B</td>
<td>-3.20±0.30</td>
<td>-3.15±0.30</td>
<td>0.09</td>
</tr>
<tr>
<td>AB vs. BA</td>
<td>-3.42±0.43</td>
<td>-2.94±0.43</td>
<td>0.20</td>
</tr>
</tbody>
</table>

5. Conclusion

PERG losses reflects both functional and anatomic loss of Retinal Ganglion Cells (RGC) as well as glial changes. PERG losses precede temporally the development of early damage of the visual field and cup/disk ratio and may be significant predictors of the evolution of the disease. Short term improvement of PERG amplitude may be expected as a measure of efficacy of neuroprotective treatments.

In this study supplementation of topical hypotensive therapy with a fixed combination of citicoline, homotaurine and vitamin E is able to significantly increase the amplitude of the PERG bioelectric potential transmitted by the optical pathways to the visual cortex in primary open-angle glaucoma with compensated IOP and early damage of the visual field and cup/disk ratio.

This short term effect could be useful in counteracting the progression of the axonal damage typical of glaucomatous optic neuropathy and could be providing a restoration of early, potentially reversible RGC and glial dysfunction.

The discontinuation of the supplement therapy was associated with an equally significant decrease in the bioelectric potential within 4 months.

In a disease involving a high risk of blindness in which the treatment is still based exclusively on reducing intraocular pressure, the chronic administration of this nutraceutical could help slow the evolution of the glaucomatous optic neuropathy.
References


