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Neuroprotective role of phosphoserine in primary open-angle glaucoma patients

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Abstract. – OBJECTIVE: To evaluate the neuroprotective role of phosphoserine (P-Ser) in primary open-angle glaucoma (POAG) patients and to compare its therapeutic effectiveness to placebo treatment.

PATIENTS AND METHODS: Fifty-one patients (24 males and 27 females) between 35 and 61 years (average 46 years ± 3.8 SD) affected by POAG were enrolled in this study. Patients were divided in two groups: group A included 28 subjects that received an oral P-Ser treatment for 12 months; and group B included 23 subjects that received an oral placebo treatment for 12 months. Complete ophthalmological examination, standard automated perimetric examination, analysis of ON fibers via scanning laser polarimetry and glaucoma staging was performed in all patients at enrolment and 1, 3, 6, and 12 months after. Statistical analysis was performed using STATA 14.0 (Collage Station, TX, USA).

RESULTS: Mean deviation (MD) and pattern standard deviation (PSD) analysis by means of 30-2 full threshold of the visual fields (VFs), retinal nerve fiber layer (RNFL) thickness by means of GDx, and IOP were considered to evaluate P-Ser therapy effectiveness in both groups. A statistically significant improvement ($p<0.05$) in VF, RNFL thickness and IOP compared to pre-treatment was found in patients in group A.

CONCLUSIONS: Our study shows a significant improvement in several variables in patients with glaucoma treated with P-Ser compared to placebo and suggests a potential neuroprotective effect of P-Ser in treating glaucoma patients in association with the traditional hypotonic topical therapy.

Key Words:
Glaucomatous optic neuropathy, Intraocular pressure, Neuroprotection.

Introduction

Glaucoma includes a group of chronic, degenerative and multifactorial pathologies characterized by peculiar anatomical-functional alterations of the optic nerve (ON) and visual field (VF). Primary open-angle glaucoma (POAG) represents the most common form of glaucoma[1].

In optic glaucomatous neuropathy, a progressive slimming down of the retinal nerve fiber layer (RNFL) occurs and is highlighted by an accentuation of the excavation of the head of the ON or disk cupping *via* pathophysiological mechanisms which are still not fully identified[2]. Intraocular pressure (IOP) increase is no longer considered a crucial factor in the definition of glaucoma and the disease is not always associated with high IOP. However, numerous studies have confirmed on large patient series the efficacy of low-intensive therapy within the sphere of glaucomatous pathology, even in presence of normal IOP. The positive action of hypotonic eye drops on the tropism of retinal structures appears to be due to a reduction of the mechanical stress exerted by the IOP either on retinal ganglion cells (RGCs) and/or on the vascular structures of the cribriform plate[3,4].

The survival of RGCs is largely influenced by the equilibrium between anti-apoptotic factors (neurotrophins) and pro-apoptotic factors, including hyperbaric stress, and accompanied by genetic and/or metabolic factors[5-10]. As a consequence of the imbalance between cellular survival mechanisms and cellular death mechanisms, a hyper-activation of the enzyme phospholipase-A2 (PLP-A2) is often observed. PLP-A2 is able to

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catabolize phosphatidylcholine (PDC), the principal RGC membrane's phospholipid, arachidonic acid and diacylglycerols which, in physiological concentrations, represent important intracellular messages[11,12]. Phosphoserine (P-Ser) is a constituent of the structural matrix of all cellular membranes and plays a fundamental role in the synthesis of neurotransmitters. It contributes to improve the functional state either of neurons of the central nervous system (CNS), amplifying cognitive activity, and of theRGCs, which augment neuro-conductance. In addition, P-Ser has been shown to stimulate the immune system and possibly improve the mood in some subjects with neurosis[13].

In the present study, we evaluated the effects of P-Ser in patients with POAG to evaluate the rationale of a neuroprotective role of the P-Ser and to compare its therapeutic effectiveness to placebo treatment.

Patients and Methods

This study was conducted according to the procedures of the Helsinki Declaration and Good Clinical Practice guidelines. Each subject signed an informed consent agreement at the beginning of the research. The protocol was approved by the Ethics Committee of Alma Mater Studiorum University of Bologna, Italy.

Inclusion criteria were: good tonometry compensation with therapy, IOP < 17 mmHg ± 2 standard deviation (SD), corrected in function of the corneal thickness according to the LALES study[2]; papillary excavation with cup/disk < 0.5 mm; 20/20 of best corrected visual acuity (BCVA) with a correction not superior to ± 4 diopters (spherical equivalent); pattern visual evoked potential (pVEP) with increased latency > 111 ms[14]; initial parametric alterations of the VF according to the glaucoma staging system (GSS)[15]; absence of concomitant neurological and/or systemic ocular pathologies. Exclusion criteria were: ocular hypertension; glaucoma with severe deficit of the VF, without therapeutic control or undergoing topical and oral maximal therapy; previous ocular surgery for cataract, glaucoma, or retinal detachment; diabetes mellitus and other systemic conditions.

Fifty-one patients with a clinical diagnosis of POAG were included in the study; all subjects were under medical topical therapy for glaucoma with a combination of beta blockers, carbonic anhydrase inhibitors, and prostaglandin analogues. After inclusion, patients were randomly divided into two groups. Group A (study group) included 28 patients, 12 males and 16 females; Group B (control group) included 23 patients, 12 males and 11 females.

At enrolment (T0) and 1 (T1), 3 (T2), 6 (T3) and 12 (T4) months after enrolment all patients underwent complete ophthalmological examination, including history, BCVA, IOP measurement using Goldmann applanation tonometry after topical anaesthetic drop application, slit-lamp biomicroscopy, mydriatic indirect fundus biomicroscopy and optic disk exam with +90 diopters Volk lens. Following ophthalmological examination, all patients underwent standard automated perimetric examination at 30-2 full threshold (Humphrey Field Analyzer, Carl Zeiss Meditec, Dublin, CA, USA), analysis of ON fibers *via* scanning laser polarimetry GDx (Glaucoma Diagnostic, Carl Zeiss Meditec Inc Dublin, CA), and glaucoma aging according to GSS guidelines[15].

A preparation of P-Ser at a concentration of 60 mg/100 ml was administered orally to patients in the study group. The oral solution was given at an elevated bioavailability in cycles of 14 consecutive days per month, for 12 months between May 2018 and May 2019. Placebo was administered to patients in control group using the same method and timepoints.

Statistical Analysis

Statistical analysis was performed using the STATA 14.0 software (Collage Station, Texas, USA). Results were presented as mean value ± SD. All data underwent statistical analysis using analysis of variance (ANOVA) for repeated measurements. A *p*-value less than 0.05 was considered statistically significant. Pairwise comparisons were performed with Bonferroni test, the mean deference was considered significant at the 0.05 level.

Results

Fifty-one patients with a diagnosis of POAG were included in this prospective randomized placebo-controlled study. Twenty-four were males (47.1%) and 27 were females (52.9%). The mean age was 46 ± 3.8 SD years (range: 35-61 years).

Mean deviation (MD) and pattern standard deviation (PSD) were separately evaluated for VF in all patients.

Table I. Values, average and SD, of POAG group A at the beginning of treatment (T0) with P-Ser and during follow-up, after 30 (T1), 90 (T2), 180 (T3), and 360 days (T4).

Follow-up	MD group A average (±SD)	PSD group A average (±SD)	RNFL group A average (±SD)	IOP group A average (±SD)
Pre-treatment (T0)	-5.49 (±0.39)	4.99 (±0.25)	411.64 (±0.50)	16.61 (±0.43)
1st month (T1)	-3.66 (±0.38)	4.9 (±0.25)	411.54 (±0.46)	15.48 (±0.16)
3rd month (T2)	-2.31 (±0.26)	4.66 (±0.26)	410.34 (±0.35)	15.42 (±0.19)
6th month (T3)	-2.27 (±0.26)	4.55 (±0.24)	410.16 (±0.31)	15.22 (±0.17)
12th month (T4)	-1.92 (±0.29)	3.51 (±0.37)	410.09 (±0.31)	15.09 (±0.17)

SD: standard deviation; POAG: primary open-angle glaucoma; P-Ser: phosphoserine; MD: mean deviation; PSD: pattern standard deviation; RNFL: retinal nerve fiber layer; IOP: intraocular pressure.

Table II. Values, average and SD, of POAG group B at the beginning of treatment (T0) with placebo and during follow-up, after 30 (T1), 90 (T2), 180 (T3), and 360 days (T4).

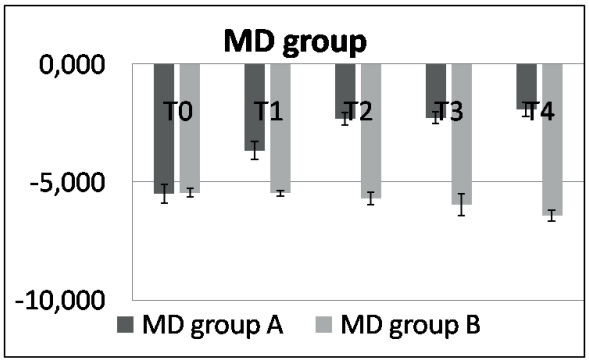
Follow-up	MD group A average (±SD)	PSD group A average (±SD)	RNFL group A average (±SD)	IOP group A average (±SD)
Pre-treatment (T0)	-5.45 (±0.18)	4.97 (±0.24)	411.52 (±0.48)	16.6 (±0.45)
1st month (T1)	-5.48 (±0.11)	5.04 (±0.25)	411.51 (±0.73)	16.48 (±0.47)
3rd month (T2)	-5.69 (±0.27)	5.33 (±0.35)	410.44 (±0.9)	16.44 (±0.45)
6th month (T3)	-5.96 (±0.46)	5.48 (±0.36)	410.38 (±0.58)	16.36 (±0.46)
12th month (T4)	-6.42 (±0.23)	5.59 (±0.34)	410.32 (±0.37)	16.28 (±0.45)

SD: standard deviation; POAG: primary open-angle glaucoma; MD: mean deviation; PSD: pattern standard deviation; RNFL: retinal nerve fiber layer; IOP: intraocular pressure.

Tables I and II show MD, PSD, RNFL, and IOP values of group A, at the beginning of treatment and during follow-up. Table III shows differences for studied variables between patients in the study group *vs.* control group.

A significant improvement ($p<0.05$) of the VF emerged from critical evaluation of the perimetric data in patients in group A compared to pre-treatment data (T0). This improvement was found at the first time point (T1; 1 month after the beginning of treatment) and remained constant during follow-up visits. Contrarily, no significant improvements ($p>0.05$) in perimetric data were found in patients in the control group (Figure 1, upper panel). A substantial stability of PSD data was found in all patients, confirming the trustworthiness of the perimetric test performed and also indicating that the damage detected from the exam is the result of a persistent localized defect (Figure 1, lower panel).

The measurement of RNFL showed a significantly greater stability ($p<0.05$) of the values in patients in group A compared to group B patients (Figure 2, upper panel). IOP measurement in pre-treatment (T0) and during follow-up showed a statistically significant reduction ($p<0.05$) of the pressure values in patients treated with P-Ser (study group) compared to patients treated with



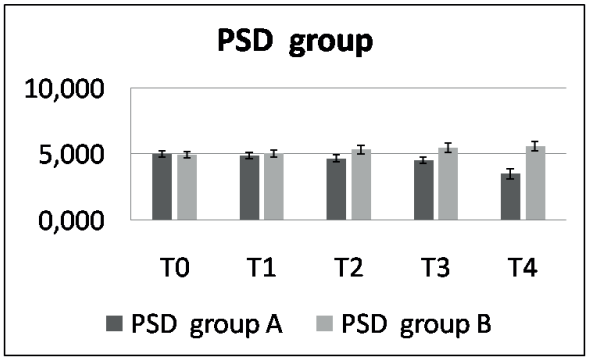


Figure 1. Evaluation of the mean deviation (MD) (upper panel) and pattern standard deviation (PSD) (lower panel) with visual fields (VFs). Primary open-angle glaucoma (POAG) patients included in A (60 mg/100 ml of phosphoserine administered in oral solution) and B (placebo) groups, at the beginning of treatment (T0) and during follow-up, after 30 (T1), 90 (T2), 180 (T3), and 365 days (T4).

Table III. Statistical significance of POAG study group A vs. control group B applied for one-way analysis of variance (ANOVA) test.

Group A vs. Group B	p	MD	PSD	RNFL	IOP
Pre-treatment (T0)	NS	0.392	0.553	0.170	0.905
1st month (T1)	< 0.05 NS (RNFL)	0.000	0.002	0.791	0.000
3rd month (T2)	< 0.05	0.000	0.000	0.000	0.000
6th month (T3)	< 0.05	0.000	0.000	0.000	0.000
12th month (T4)	< 0.05	0.000	0.000	0.000	0.000

The statistical significance was attested at a probability of $p < 0.05$. Value significantly higher by one-way ANOVA test. POAG: primary open-angle glaucoma; MD: mean deviation; PSD: pattern standard deviation; RNFL: retinal nerve fiber layer; IOP: intraocular pressure; NS: not significant. Values at the beginning of treatment (T0) and during follow-up, after 30 (T1), 90 (T2), 180 (T3), and 360 days (T4).

placebo (Figure 2, lower panel). None of the patients treated with P-Ser showed adverse effects to the treatment.

Discussion

In the present study, we evaluated the neuroprotective role of P-Ser compared to placebo in patients with POAG under maximal IOP lowering therapy and found a significant improvement in ON function in treated patients. Glaucoma is an age-related progressive optic neuropathy which shares pathophysiological features with CNS degenerative diseases as Huntington's disease, Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis (ALS)[16,17]. Medical therapies targeted on protection of the ON and on prevention of RGC death are therefore thought to play an increasingly important role in the future of glaucoma treatment and are supported by the preliminary findings reported in the present study.

The concept of neuroprotection was born in 1990s and was proposed as an additional or alternative therapy to IOP lowering[18]. In the last years several molecules have been discovered to present a compelling neuroprotective role: neurotrophins, such as ciliary-derived neurotrophin factor, brain-derived neurotrophic factor, galantamine, brimonidine, memantine, antioxidant and free radical scavengers, ginkgo biloba extract, nitric oxide synthase inhibitor[19-25]. The survival of RGCs is widely dependent on the equilibrium between anti-apoptotic, or neurotrophins, and pro-apoptotic molecules. Consequently, an imbalance between these two groups of factors is able to determine a cascade of events including hyper-activation of PLP-A2[26-29].

P-Ser is a membrane component which present a similar structure to glutamate. Klunk et al[30] demonstrated that P-Ser could bind to three subtypes of Glu receptors: the a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/quisqualic acid, kainic acid and NMDA-selective subtypes. Two pathways are known to play a significant role in serine synthesis, a "phosphorylated" and a "non-phosphorylated" pathway. In the first pathway, the intermediate phosphoglycerate is turned into phosphohydroxypyruvate which is then con-

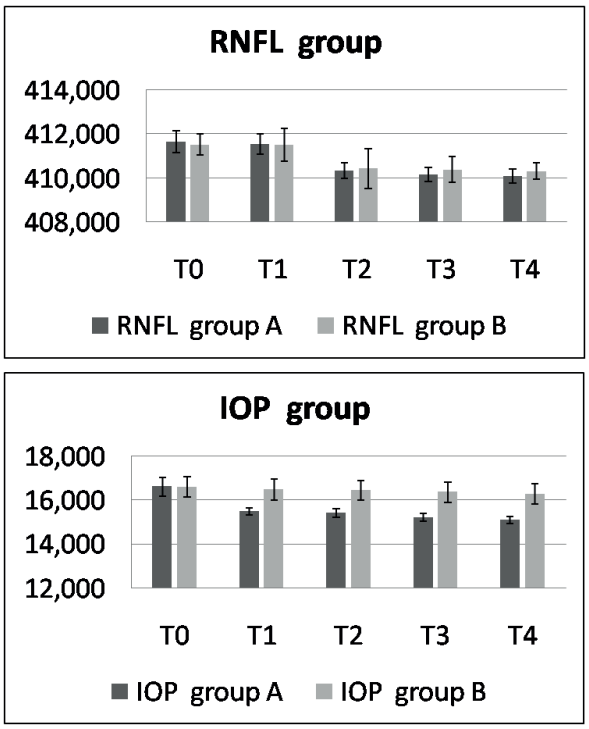


Figure 2. Evaluation of the variations of the retinal nerve fiber layer (RNFL) (upper panel) and intraocular pressure (IOP) (lower panel). Primary open-angle glaucoma (POAG) patients included in A (60 mg/100 ml of phosphoserine administered in oral solution) and B (placebo) groups, at the beginning of treatment (T0) and during follow-up, after 30 (T1), 90 (T2), 180 (T3), and 365 days (T4).

verted into P-Ser by phosphohydroxypyruvuvate aminotransferase. P-Ser is then metabolised to L-serine by P-Ser phosphatase. The phosphorylated pathway's enzymes are strongly expressed in different tissues as the brain and kidneys where L-serine represents a crucial intermediate in the synthesis of gluconeogenesis molecules as pyruvate and hydroxypyruvate. Furthermore, cysteine and glycine, the precursors of glutathione, derive from L-serine metabolism[31]. Moreover, phosphatidylserine represents an essential molecule in the apoptosis signalling pathway and an important precursor of sphingolipids which are key membrane and myelin components[32].

L-serine is known to have trophic effects on neurons in culture: when added at physiological concentrations into neuron cultures, it shows compelling effect on axon length[33]. Neurons in cultures use exogenous L-serine for the synthesis of L-serine-derived phospholipids[33]; the biosynthesis of these phospholipids is severely decreased in the absence of serine or glycine[34]. Different neuromodulators derived from serine as glycine and D-serine have a quite similar action to that of glutamate, but on glial cells. These cells contribute to create a peculiar microenvironment suitable for neuron functioning, confirming that serine homeostasis is crucial in maintaining this homeostasis condition. Glycine is known to represent the major inhibitory neurotransmitter in the CNS and is an agonist of NMDA receptors[35]. Snyder and Kim hypothesized that glutamate could both bind to NMDA receptors and determine D-serine release from nearby astrocytes *via* non-NMDA receptors; both glutamate and D-serine could activate the post-synaptic NMDA receptor complex[36]. It seems that glycine and D-serine are co-agonists of the NMDA receptors, so they could be closely related in activation of this receptor complex, especially during the development of the foetal brain[37]. Furthermore, recent studies highlighted a neuroprotective role of serine against β-N-methylamino-L-alanine (L-BMAA), a neurotoxic amino acid that is able to activate endoplasmic reticulum (ER) stress[38] and that is linked to neurodegenerative diseases as AD and Guamanian ALS/parkinsonism dementia complex (ALS/PDC)[39].

ER-stress is due to a lack of homeostasis in the ER and has been linked to several neurodegenerative diseases, such as ALS and AD[40,41]. Several mechanisms are involved in L-BMAA neurotoxicity: from bicarbonate L-BMAA obtains a carbamate, which determines excitotoxicity as an agonist of NMDA receptor, AMPA/kainite receptor, and metabotropic glutamate receptor 5 (mGluR5)[42-44]. As a consequence, phosphatase 2A (PP2A) decreases determining tau hyper-phosphorylation, a typical sign of AD[42,43].

L-Serine showed a protective action against L-BMAA induced neurotoxicity both *in vitro* and *in vivo*[39-45]. Phase I of clinical trial of safety of L-serine for patients affected by ALS/motor neuron disease (ALS/MND) highlighted a significant slowing of disease progression after oral L-serine administration[46].

In light of this, it is clear from the literature that serine synthesis and metabolism are important in brain function and, as shown in our study, in ON physiopathology.

Conclusions

Our study showed a significant improvement in several variables in patients with glaucoma treated with P-Ser compared to placebo and suggests a potential neuroprotective effect of P-Ser in treating glaucoma patients in association with the traditional hypotonic topical therapy. Based on these results, it could be hypothesized that P-Ser, associated to a correct topical therapy, could determine a decrease of glaucoma progression. To the best of our knowledge, this is the first study that evaluates the neuroprotective role of P-Ser in patients affected by glaucoma. Therefore, it is necessary to clarify which are the main metabolic pathways of P-Ser at the level of the ON; further studies are necessary to confirm our preliminary results and open possible future uses of P-Ser.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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Authors' Contributions

SZC, ST, VP, MR, AG and FF designed the study. SZC and ML performed the experiments, and LS and FF analyzed the data. All authors interpreted the results and produced and approved the final manuscript.

Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of Alma Mater Studiorum University of Bologna, Italy.

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