GINKGO BILOBA, SELENIUM, OMEGA-3, AND VITAMINS D AND E ON INTERLEUKIN-6 AND SUPEROXIDE DISMUTASE IN MAJOR NEUROCOGNITIVE DISORDER

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GINKGO BILOBA, SELENIUM, OMEGA-3, AND VITAMINS D AND E ON INTERLEUKIN-6 AND SUPEROXIDE DISMUTASE IN MAJOR NEUROCOGNITIVE DISORDER (Abstract): Major neurocognitive disorder is characterized by oxidative stress and neuroinflammation, two interconnected processes that current symptomatic therapies do not address. **Materials and methods:** This study evaluated the effect of a combined antioxidant intervention, added to standard antidementia therapy, on a proinflammatory marker (interleukin-6, IL-6) and an antioxidant-defense marker (superoxide dismutase, SOD). In this prospective, open-label study, 60 patients with mild-to-moderate major neurocognitive disorder were randomized, using a computer-generated sequence, to standard therapy alone (donepezil and memantine; n = 30) or to standard therapy plus a daily antioxidant combination comprising Ginkgo biloba extract, selenium, omega-3 fatty acids, and vitamins E and D (n = 30). **Results**: Serum IL-6 and SOD activity were assessed at baseline and after three months; 49 patients completed the study. The two groups were comparable at baseline. In the intervention group, IL-6 decreased significantly (6.8 ± 2.1 to 4.9 ± 1.7 pg/mL, −28%, $p < 0.001$) and SOD activity increased significantly (1180 ± 240 to 1420 ± 270 U/mL, +20%, $p < 0.001$), whereas standard therapy alone produced no significant change in either marker; at three months, both markers differed significantly between groups ($p < 0.001$). **Conclusions:** The combined antioxidant intervention thus produced measurable, concordant effects on the interconnected processes of neuroinflammation and oxidative stress. Given the open-label design, modest sample size and short follow-up, these preliminary findings require confirmation in larger, blinded, biomarker-guided trials incorporating baseline stratification and clinical endpoints. **Keywords:** MAJOR NEUROCOGNITIVE DISORDER, OXIDATIVE STRESS, NEUROINFLAMMATION, ANTIOXIDANTS, INTERLEUKIN-6, SUPEROXIDE DISMUTASE.

INTRODUCTION

Major neurocognitive disorder, the DSM-5 term for dementia, is among the leading causes of disability and dependency in older adults, and its prevalence is rising rapidly with population ageing (1).

Current pharmacological options - cholinesterase inhibitors and memantine - are symptomatic and produce only modest benefits, without altering the underlying neurodegenerative process (2). This limitation has sustained interest in complementary strategies directed at the fundamental pathogenic mechanisms of the disease, among which oxidative stress and neuroinflammation occupy a central position.

Oxidative stress, defined as an imbalance between reactive oxygen species and antioxidant defenses, is an early event in Alzheimer's disease, detectable from the stage of mild cognitive impairment (3,4). The brain is particularly vulnerable owing to its high oxygen consumption, its lipid-rich membranes and its relatively low antioxidant enzyme content (3). Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion, constitutes the first line of enzymatic antioxidant defense and is widely used as a marker of antioxidant capacity. In parallel, neuroinflammation is now recognized as an active driver of neurodegeneration rather than an epiphenomenon (5); interleukin-6 (IL-6), a pleiotropic proinflammatory cytokine, is consistently elevated in Alzheimer's disease and correlates with the severity of cognitive decline (6). Oxidative stress and neuroinflammation are mutually reinforcing, forming a self-perpetuating vicious cycle that sustains neuronal injury (3,5).

Individual antioxidant compounds have a solid mechanistic rationale but have yielded predominantly modest or inconsistent clinical results (7). A plausible explanation is that an isolated antioxidant, once oxidized and not regenerated, may itself become pro-oxidant, which argues for combined formulations whose components act complementarily within the antioxidant network (8). This rationale underlies multinutrient combinations such as that evaluated in the LipiDiDiet trial (9). However, most studies have relied on clinical endpoints alone, without verifying whether the intervention actually modulates the targeted biological processes - a gap that is relevant given that peripheral oxidative and inflammatory markers are readily measurable in serum (10).

The present study evaluated the effect of a combined antioxidant intervention - Ginkgo biloba, selenium, omega-3 fatty acids, and vitamins D and E - added to standard antidementia therapy, on a proinflammatory marker (IL-6) and an antioxidant-defense marker (SOD), in patients with major neurocognitive disorder.

MATERIALS AND METHODS

Study design and participants

This prospective, comparative study was conducted over three months at Socola Institute of Psychiatry Iasi, between February 2024 and April 2024. The protocol was approved by the Socola Institute of Psychiatry's Ethics Committee (approval no. 30525/06.11.2023) and "Gr. T. Popa" University of Medicine and Pharmacy's Ethics Committee (approval no. 380/17.01.2024), and conducted in accordance with the Declaration of Helsinki; all participants or their legal representatives provided written informed consent.

Eligible patients were adults aged ≥60 years with major neurocognitive disorder diagnosed according to DSM-5 criteria (11), of mild-to-moderate severity (Mini-Mental State Examination [MMSE] score 10-24) (12), on stable standard antidemen-

tia therapy. Exclusion criteria were: current use of antioxidant or anti-inflammatory supplements; acute or chronic inflammatory, infectious or autoimmune disease; recent (<3 months) myocardial infarction or stroke; severe hepatic or renal impairment; active malignancy; and ongoing anticoagulant therapy.

Sixty patients were enrolled. Patients were randomly allocated to two parallel groups in a 1: 1 ratio using a computer-generated randomization sequence. The study was open-label, with no blinding of participants or investigators. The control group (n = 30) received standard therapy alone - donepezil 10 mg/day and memantine 20 mg/day. The intervention group (n = 30) received the same standard therapy plus a combined antioxidant supplement, administered daily, comprising: standardized Ginkgo biloba extract (EGb 761) 240 mg, selenium 100 µg (as L-selenomethionine), omega-3 fatty acids providing eicosapentaenoic acid and docosahexaenoic acid 660 mg, natural vitamin E (RRR-α-tocopherol) 100 IU, and vitamin D3 (cholecalciferol) 2000 IU.

Outcome measures and laboratory analysis

The primary outcomes were serum IL-6 concentration and SOD activity, assessed at baseline (T0) and after three months (T1). Venous blood was collected in the morning after overnight fasting, centrifuged within 30 minutes, and serum was stored at −80 °C until analysis. IL-6 was measured by enzyme-linked immunosorbent assay (Human IL-6 Conferma® ELISA, Sigma-Aldrich) and expressed in pg/mL; SOD activity was determined by a commercial spectrophotometric assay (MAK528, Sigma-Aldrich) and expressed in U/mL. All samples were analyzed in duplicate. Cognitive status was characterized at baseline using the MMSE.

Statistical analysis

Data were analyzed using *SPSS version 24*. Normality of distributions was assessed with the Shapiro-Wilk test. Continuous variables are expressed as mean ± standard deviation. Within-group changes between T0 and T1 were compared using the paired t-test or the Wilcoxon signed-rank test, and between-group differences using the independent-samples t-test or the Mann-Whitney U test, as appropriate. Categorical variables were compared with the chi-square test. A two-tailed p value <0.05 was considered statistically significant.

RESULTS

Of the 60 enrolled patients, 49 completed the three-month study: 24 in the control group and 25 in the intervention group. Eleven patients withdrew (6 control, 5 intervention); reasons were withdrawal of consent, loss to follow-up, and intercurrent illness, with no significant difference in attrition between groups ($p = \leq 0.05$). The two groups were comparable at baseline with respect to age, sex distribution, and MMSE score (all $p > 0.05$) (tab. I).

At baseline, serum IL-6 and SOD activity did not differ significantly between groups (tab. II). In the intervention group, IL-6 decreased significantly from 6.8 ± 2.1 pg/mL at T0 to 4.9 ± 1.7 pg/mL at T1, corresponding to a reduction of approximately 28% ($p < 0.001$). In the control group, IL-6 showed only a small, non-significant change (6.7 ± 2.0 to 6.4 ± 1.9 pg/mL; $p = 0.34$). At T1, IL-6 was signifi-

cantly lower in the intervention group than in the control group ($p < 0.001$).

SOD activity increased significantly in the intervention group, from 1180 ± 240 U/mL at T0 to 1420 ± 270 U/mL at T1, an increase of approximately 20% ($p < 0.001$). In the control group, SOD activity remained essentially unchanged (1190 ± 245 to 1235 ± 255 U/mL; $p = 0.27$). At T1, SOD activity was significantly higher in the intervention group than in the control group ($p < 0.001$) (tab. II).

Thus, over three months, the addition of the combined antioxidant intervention to standard therapy was associated with a significant reduction in the proinflammatory marker IL-6 and a significant increase in the antioxidant-defense marker SOD, whereas standard therapy alone produced no significant change in either marker.

TABLE I.

Baseline characteristics of the study groups

Characteristic	Control group (n = 24)	Intervention group (n = 25)	p
Age (years), mean ± SD	72.8 ± 7.1	72.0 ± 6.5	0.68
Sex (F/M), n	15/9	11/14	1.00(Fisher)
MMSE score, mean ± SD	18.4 ± 3.3	18.8 ± 3.1	0.66

SD - standard deviation; MMSE - Mini-Mental State Examination.

TABLE II.

Serum IL-6 and SOD activity at baseline (T0) and after three months (T1)

Parameter	Group	T0 (mean ± SD)	T1 (mean ± SD)	Δ (%)	p (T0 *vs.* T1)
IL-6 (pg/mL)	Control	6.7 ± 2.0	6.4 ± 1.9	−4	0.87
	Intervention	6.8 ± 2.1	4.9 ± 1.7	−28	<0.001
SOD (U/mL)	Control	1190 ± 245	1235 ± 255	+4	0.89
	Intervention	1180 ± 240	1420 ± 270	+20	<0.001

IL-6 - interleukin-6; SOD - superoxide dismutase; SD - standard deviation.
Between-group comparison at T1: $p < 0.001$ for both parameters.

DISCUSSION

In this study, the addition of a combined antioxidant intervention to standard antidementia therapy was associated, over three months, with a significant reduction in serum IL-6 and a significant increase in SOD activity, whereas standard therapy alone produced no significant change in either marker. To our knowledge, few studies have evaluated the effect of a multi-

component antioxidant formulation on both an inflammatory and an antioxidant-defense marker in patients with established major neurocognitive disorder, most previous trials having relied on clinical endpoints alone (7).

The observed changes are biologically coherent and mutually consistent. The rise in SOD activity indicates a strengthening of enzymatic antioxidant defense, while the fall in IL-6 reflects attenuation of the pro-inflammatory state. Because oxidative stress and neuroinflammation are linked in a self-reinforcing cycle - reactive oxygen species activate nuclear factor-κB, which drives cytokine synthesis, and cytokines in turn promote further oxidative stress (5), an intervention acting on one arm would be expected to influence the other. The parallel movement of the two markers in opposite but concordant directions is compatible with this coupling.

These findings are consistent with the mechanistic properties of the individual components. Standardized Ginkgo biloba extract increases the activity of endogenous antioxidant enzymes, including SOD, and attenuates microglial cytokine release (13). Selenium supports the selenium-dependent glutathione peroxidase situated immediately downstream of SOD, and exerts anti-inflammatory effects through modulation of nuclear factor-κB (14). Omega-3 fatty acids give rise to specialized pro-resolving mediators that actively downregulate pro-inflammatory cytokines (15), while vitamin D exerts immunomodulatory effects and promotes the expression of antioxidant enzymes (16). This mechanistic convergence provides a rationale for the combined approach: rather than relying on a single high-dose agent, the formulation combines several compounds with complementary actions at nutritional doses.

The combined strategy also addresses a recognized limitation of single-antioxidant therapy. An isolated antioxidant, once oxidized and not regenerated, may itself acquire pro-oxidant properties, which may partly explain the modest results of antioxidant monotherapies (8). Complementary combinations, in which components regenerate one another within the antioxidant network, provide a mechanistic argument for multi-component formulations, as exemplified by the multinutrient combination evaluated in the LipiDiDiet trial (9). The present results, by demonstrating measurable biological modulation, provide preliminary support for this rationale in patients with established disease - a population in which such combinations have seldom been studied.

Several limitations must be acknowledged. First, the study was open-label, without blinding of participants or investigators, which may introduce bias; although biomarker outcomes are objective and less susceptible to observer bias than clinical scales, an open-label design cannot be regarded as definitive. Second, the sample size was modest and the follow-up short (three months), so that the durability of the observed changes and their translation into clinical benefit remain undetermined. Third, the combined design does not allow the contribution of each component to be isolated. Fourth, and importantly, the baseline oxidative and inflammatory status of participants was not used as a selection criterion; since antioxidant supplementation is most likely to benefit individuals with an underlying imbalance (8), the unstratified design may have diluted the ef-

fect. Fifth, IL-6 and SOD are peripheral markers and do not necessarily reflect central nervous system processes, although peripheral oxidative and inflammatory changes have been documented in this population (10). Finally, cognitive and functional outcomes were not the focus of the present analysis and will be reported separately.

Notwithstanding these limitations, the study has notable strengths: a prospective comparative design with computer-generated randomization, the simultaneous assessment of an inflammatory and an antioxidant marker, and the evaluation of an accessible, low-cost intervention added to standard care in a real-world clinical setting. The findings suggest that combined antioxidant supplementation can produce measurable biological effects on oxidative and inflammatory markers in major neurocognitive disorder, and support the conduct of larger, blinded, biomarker-guided trials incorporating baseline stratification and clinical endpoints.

CONCLUSIONS

In patients with major neurocognitive disorder, the addition of a combined antioxidant intervention (Ginkgo biloba, selenium, omega-3 fatty acids, and vitamins D and E) to standard antidementia therapy was associated, over three months, with a significant reduction in serum IL-6 and a significant increase in SOD activity, whereas standard therapy alone produced no significant change. These concordant changes indicate that the combined formulation can exert measurable effects on the interconnected processes of neuroinflammation and oxidative stress. Given the open-label design, the modest sample size and the short follow-up, these preliminary findings require confirmation in larger, blinded, biomarker-guided trials incorporating baseline stratification and clinical endpoints, in order to establish whether the observed biological modulation translates into meaningful clinical benefit.

CONFLICT OF INTEREST AND FUNDING

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