Phosphatidylserine Containing Omega-3 Fatty Acids May Improve Memory Abilities in Nondemented Elderly Individuals with Memory Complaints: Results from an Open-Label Extension Study

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Key Words
Phosphatidylserine · Omega-3 fatty acids · Docosahexaenoic acid · Cognitive decline · Memory complaints

Abstract
Background: The present study is an open-label extension (OLE) aimed at evaluating the effect of 100 mg/day of phosphatidylserine enriched with docosahexaenoic acid (PS-DHA) on cognitive performance in nondemented elderly individuals with memory complaints. Methods: From the participants who completed the core study, 122 continued with a 15-week OLE. Efficacy was assessed using a computerized tool and the Clinical Global Impression of Change (CGI-C) rating scale. Results: A significant improvement in sustained attention and memory recognition was observed in the PS-DHA naïve group, while the PS-DHA continuers maintained their cognitive status. Additionally, a significant improvement in CGI-C was observed in the naïve group. Conclusions: The results demonstrate that consumption of 100 mg/day of PS-DHA might be associated with improving or maintaining cognitive status in elderly subjects with memory complaints.

Introduction

Phosphatidylserine (PS) is one of the major phospholipids in mammalian cell membranes and it plays important roles in dynamic membrane functions [1, 2]. With aging, neural membrane fluidity is compromised due to the increased presence of cholesterol, a low incor-
poration rate and decreased levels of total polyunsaturated fatty acids, blockages to phospholipid pathways, and increases in free radicals, resulting in oxidative stress [3]. The brain is one of the richest organs in lipid content, and its structure and function have been shown to be influenced by nutrients [4]. Unfavorable changes in the brain phospholipid levels (lipid imbalance) could lead to different pathogenic processes [5], as demonstrated in various neuronal conditions. Clinical trials, conducted in the early 1990’s, indicated that consumption of 100–300 mg/day of PS extracted from bovine cortex (BC-PS) plays important roles in the support of mental functions in the aging brain [6–8]. BC-PS was found to be enriched with docosahexaenoic acid (DHA) [9]. However, due to safety concerns of potential contamination by bovine spongiform encephalopathy prions, BC-PS is no longer available. Alternatives such as soybean-derived PS are considered a safe alternative; however, soybean-derived PS does not contain DHA, and clinical studies have demonstrated inconclusive efficacy results [10, 11]. We have recently reported on a safe sourced PS enriched with DHA (PS-DHA) to have beneficial effects in nondemented elderly individuals with memory complaints [12]. In this study, subjects with memory complaints were randomized to receive either PS-DHA or placebo for 15 weeks. Efficacy measures, assessed at baseline and endpoint, included the Rey Auditory Verbal Learning Test, Rey Complex Figure Test, and others. At the end of a 15-week, double-blinded, placebo-controlled study, verbal immediate recall was significantly improved in the PS-DHA group compared to the placebo group. Interestingly, post hoc analysis revealed that participants with a relatively good cognitive performance at baseline were most likely to benefit. The safety profile of PS-DHA following a 30-week administration was also reported [13]. Briefly, PS-DHA was found to be safe and well tolerated, with no significant side effects.

Here, we report the efficacy results of a 15-week, open-label extension (OLE) study that followed the double-blind, placebo-controlled phase previously described. In this extension, the participants consumed 100 mg/day of PS-DHA.

**Methods**

**Study Design and Participants**

Detailed methods of the 15-week, double-blind, placebo-controlled core study (clinicaltrials.gov identifier: NCT00437983) have been published previously [12]. Briefly, participants were recruited through advertisements in senior citizens homes, hospitals, and newspapers. A total of 157 nondemented individuals with memory complaints met the inclusion criteria and were assigned to the study groups according to a computerized randomization process, receiving 300 mg/day of PS-DHA or identical-looking placebo (cellulose). In total, 131 participants completed the double-blind, placebo-controlled phase. The completers were invited to continue with a 15-week OLE (without breaking patients’ blinded treatment code), and 122 participants agreed to continue. During the OLE, all participants received 100 mg/day of PS-DHA, providing 100 mg of PS and an equivalent amount of 26 mg of DHA+EPA (eicosapentaenoic acid; DHA/EPA ratio 3:1). Participants who received 300 mg/day of PS-DHA during the double-blind phase and continued to receive 100 mg/day of PS-DHA during the OLE are hereafter referred to as continuers (n = 61), while participants who received placebo during the double-blind phase and switched to 100 mg/day of PS-DHA during the OLE are hereafter referred to as naïve (n = 61). PS-DHA (Vayacog®) was supplied by Enzymotec Ltd., Migdal HaEmeq, Israel. For treatment adherence monitoring, the participants returned all treatment packs at the study end point, and adherence was calculated from the number of the remaining capsules.

The study was conducted according to the principles of the Declaration of Helsinki and good clinical practice. The protocol was approved by the Ethics Committee of the Sourasky Medical Center, Tel Aviv, Israel, and all volunteers gave written informed consent prior to participation.

**Assessments**

Efficacy measurements included both objective reports and subjective measurements, assessed at the end of the double-blind phase (baseline of the OLE) and at the end of the OLE (after 15 weeks). As objective
measurements, a computerized neuropsychological assessment tool, NexAde™, was used [14]. This software consists of seven separate tasks: symbol spotting, pattern identification, pattern recall, digit-symbol substitution, digits span forward, digits span backward, and delayed pattern recall. Based on the results obtained in the single tasks, cognitive composite scores are calculated for focused attention, sustained attention, memory recognition and recall, visuospatial learning, spatial short-term memory, and a final score, which summarizes all composite score results. All tasks are computer-controlled [14]. The Clinical Global Impression of Change (CGI-C) rating scale was used to obtain subjective perceptions of improvement. Using the participants’ cognitive status impression at the beginning of the OLE as a reference, the evaluator interviewed the participants at the end of the OLE in order to obtain an impression of change during the OLE period. The global improvement score ranges from 1 = ‘very much improved’, through 4 = ‘no change’, to 7 = ‘very much worse’. The interviewer had no access to the cognitive test scores and adverse event reports obtained as part of the protocol. To examine the differences between the treatment groups in the assessment of global change, participants who experienced an improvement (scores 1–3) on the last visit were classified as ‘improved’ over the treatment period. Otherwise, participants were classified as ‘unchanged’ (score 4) or as ‘worse’ (scores 5–7).

Safety evaluation included physical examination, blood pressure, heart rate, and weight. Adverse events were also monitored.

**Statistical Analysis**

Results are expressed as mean ± standard error (SE). Student’s t test for independent samples was used to evaluate differences in demographic and baseline continuous variables. Pearson’s χ² test was used for the analysis of categorical variables and CGI-C. Data analysis was performed according to participants’ randomization to treatment or placebo in the preceding double-blind phase. Within-group analysis was tested for the naïve and continuers groups, using paired Student’s t test analysis. All statistical tests were two-tailed, and significance was set at a level of 0.05. SAS statistical package (version 9.1) was used for all analyses.

**Results**

**Participants**

A flow diagram of the study participants throughout the OLE is presented in figure 1.

Of the 122 participants enrolled in the OLE, 61 were continuers and 61 were naïve. One participant from the PS-DHA naïve group was excluded from the study due to protocol violation. Baseline characteristics of the study participants who completed the OLE are
presented in table 1. No significant differences in demographic or cognitive status were observed between the two study groups. High treatment compliance during the open label phase (<90%) was observed in both groups.

**Efficacy Assessment**

**Neurological Computerized Cognitive Assessment Tool**

Following 15 weeks of open label treatment with PS-DHA, no significant change in cognitive abilities was observed in the continuers group (fig. 2a). A different pattern was observed in the naïve group, where the results showed an improvement in six out of seven tested parameters, reaching the statistically significant level (p < 0.05) in sustained attention, memory recognition, and in the cognitive assessment final score (fig. 2b).

**Clinical Global Impression of Change**

Within the continuers group, the number of participants who were judged as clinically improved at the end of the double-blind phase was 25 (38%), similar to the number of participants who were judged as clinically improved at end of the OLE (24, 40%). Sixteen participants out of the 25 clinically improved subjects at the end of the double-blind phase were also judged as clinically improved during the OLE, while 7 participants maintained their improvement and reported no further change during the OLE.

Within the naïve group, the number of participants who were judged as clinically improved at the end of the double-blind phase was 18 (28%). A significantly higher number of participants (26, 44%; p = 0.019) was judged as clinically improved at the end of the OLE. Fourteen participants out of the 18 clinically improved subjects at the end of the double blind phase were also judged as clinically improved during the OLE, while 2 participants maintained their improvement and reported no further change during the OLE.
Safety

Adverse events and the safety report have been published previously [13]. Briefly, a reduction in resting diastolic blood pressure and a slight weight gain were observed among the continuers. While only three adverse events, classified by the study physicians as related
or probably related to the study treatment (gastrointestinal discomfort, headache and tenesmus), were reported in the continuers group, no related or probably related side effects were reported in the naïve group.

**Discussion**

This OLE study followed a 15-week, double-blind, placebo-controlled study of PS-DHA consumption at a dose of 300 mg/day. During the double-blind study, the PS-DHA group showed a significant improvement in memory (immediate recall) in nondemented subjects with memory complaints. Post hoc analysis suggested that participants with a relatively good cognitive performance at baseline responded better to the treatment [12]. During the 15-week OLE, consumption of 100 mg/day of PS-DHA was associated with a sustained cognitive status of participants previously treated with 300 mg/day of PS-DHA, while naïve participants attained a significant improvement in sustained attention and memory.

The tested marine-derived PS-DHA is considered safe [13], without any contamination concerns for PS derived from bovine brain. Consumption of 100 mg/day of PS-DHA was well tolerated, supported by the absence of any significant adverse events and by only a single exclusion during this extension phase.

The findings of this OLE study add to the accumulating data that support the positive effect of PS containing omega-3 fatty acids on cognitive performance. In previous studies, administration of 200–300 mg/day of PS containing omega-3 fatty acids was found to have beneficial effects, purely symptomatic or neuroprotective [9, 15–19] ones, on various types of cognitive impairment, ranging from age-associated cognitive decline to Alzheimer’s disease [7, 8, 20–24].

In a double-blind, placebo-controlled study, administration of BC-PS to patients with age-associated memory impairment improved their performance related to learning and memory tasks of daily life [8]. Geriatric patients treated with BC-PS significantly improved in behavioral and cognitive parameters [6]. In cognitively impaired populations, the measurement of the regional cerebral metabolic rate for glucose in AD patients using positron emission tomography and ^18^F-2-fluoro-2-deoxy-D-glucose as well as electrophysiological changes with electroencephalography indicated that BC-PS treatment has an effect on brain function [18, 25]. These changes were further correlated with neuropsychological improvements [7, 21].

The results of this OLE show for the first time that 100 mg/day of PS-DHA might significantly improve cognitive abilities in nondemented elderly individuals with memory complaints (naïve) and may preserve the effect observed following administration of 300 mg/day of PS-DHA during the double-blinded phase (continuers). These results, together with previous findings reported for BC-PS, justify further studies evaluating the effect of PS-DHA also in more severely affected populations.

While the findings obtained throughout this OLE study are encouraging, we acknowledge certain limitations. One limitation of the current study is its open-label nature and the absence of a placebo-controlled group; therefore, there is a risk of bias in the interpretation of the study results. Nonetheless, the fact that a significant improvement was observed only in the naïve group (the subjects were not informed that they have previously been in the placebo arm) suggests that the effect is genuine and not derived from the placebo or learning effect. This was further supported by the fact that the significant improvement was observed in both the objective and the subjective measurements (computerized tool and CGI-C, respectively).

To conclude, the current OLE suggests that consumption of 100 mg/day of PS-DHA might maintain or improve cognitive status in subjects with memory complaints. These preliminary results are encouraging and could assist in planning an extended, double-blind, placebo-controlled study to further establish the efficacy of this dose.
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References

1. Vance JE, Steenbergen R: Metabolism and functions of phosphatidylserine. Prog Lipid Res 2005;44:207–234.
2. Mozzi R, Buratta S, Goracci G: Metabolism and functions of phosphatidylserine in mammalian brain. Neurochem Res 2003;28:195–214.
3. Yehuda S, Rabionovitz S, Carasso RL, Mostofsky DI: The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. Neurobiol Aging 2002;23:843–853.
4. Turner J: Your brain on food: a nutrient-rich diet can protect cognitive health. Generations 2011;35:99–116.
5. Kosicek M, Hecimovic S: Phospholipids and Alzheimer’s disease: alterations, mechanisms and potential biomarkers. Int J Mol Sci 2013;14:1310–1322.
6. Cenacchi T, Bertoldin T, Farina C, Fiori MG, Crepaldi G: Cognitive decline in the elderly: a double-blind, placebo-controlled multicenter study on efficacy of phosphatidylserine administration. Aging 1993;5:123–133.
7. Crook T, Petrie W, Wells C, Massari DC: Effects of phosphatidylserine in Alzheimer’s disease. Psychopharmacol Bull 1992;28:61–66.
8. Crook TH, Tinklenberg J, Vesavage J, Petrie W, Nunzi MG, Massari DC: Effects of phosphatidylserine in age-associated memory impairment. Neurology 1991;41:644–649.
9. Kim HY, Akbar M, Kim YS: Phosphatidylserine-dependent neuroprotective signaling promoted by docosahexaenoic acid. Prostaglandins Leukot Essent Fatty Acids 2010;82:165–172.
10. Schreiber S, Kampf-Sherf O, Gofine M, Kelly D, Oppenheim Y, Lerner B: An open trial of plant-source derived phosphatidylserine for treatment of age-related cognitive decline. Isr J Psychiatry Relat Sci 2000;37:302–307.
11. Jorissen BL, Brouns F, Van Boxtel MP, Ponds RW, Verhey FR, Jolles J, Riedel WJ: The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment. Nutr Neurosci 2001;4:121–134.
12. Vakhapova V, Cohen T, Richter Y, Herzog Y, Korczyn AD: Phosphatidylserine containing omega-3 fatty acids may improve memory abilities in non-demented elderly with memory complaints: a double-blind placebo-controlled trial. Dement Geriatr Cogn Disord 2010;29:467–474.
13. Vakhapova V, Richter Y, Cohen T, Herzog Y, Korczyn AD: Safety of phosphatidylserine containing omega-3 fatty acids in non-demented elderly: a double-blind placebo-controlled trial followed by an open-label extension. BMC Neurol 2011;11:79.
14. Aharonson V, Korczyn AD: Human-computer interaction in the administration and analysis of neuropsychological tests. Comput Methods Programs Biomed 2004;73:43–53.
15. Dvoiriantchikova G, Agudelo C, Hernandez E, Shestopalov VI, Ivanov D: Phosphatidylserine-containing liposomes promote maximal survival of retinal neurons after ischemic injury. J Cereb Blood Flow Metab 2009;29:1755–1759.
16. Hashioka S, Han YH, Fujii S, Kato T, Monji A, Utsumi H, Sawada M, Nakanishi H, Kanba S: Phosphatidylserine and phosphatidylcholine-containing liposomes inhibit amyloid beta and interferon-gamma-induced microglial activation. Free Radic Biol Med 2007;42:945–954.
17. Bonetti AC, Bellini F, Calderini G, Galbiati E, Toffano G: Age-dependent changes in the mechanisms controlling prolactin secretion and phosphatidylinositol turnover in male rats: effect of phosphatidylserine. Neuroendocrinology 1987;45:123–129.
18. Heiss WD, Kessler J, Mielke R, Szelies B, Herholz K: Long-term effects of phosphatidylserine, pyritinol, and cognitive training in Alzheimer’s disease. A neuropsychological, EEG, and PET investigation. Dementia 1994;5:88–98.
19. Blokland A, Honig W, Brouns F, Jolles J: Cognition-enhancing properties of subchronic phosphatidylserine (PS) treatment in middle-aged rats: comparison of bovine cortex PS with egg PS and soybean PS. Nutrition 1999;15:778–783.
20. Amaducci L, Crook TH, Lippi A, Bracco L, Baldereschi M, Latorraca S, Piersanti P, Tesco G, Sorbi S: Use of phosphatidylserine in Alzheimer’s disease. Ann NY Acad Sci 1991;640:245–249.
21. Amaducci L: Phosphatidylserine in the treatment of Alzheimer’s disease: results of a multicenter study. Psychopharmacol Bull 1988;24:130–134.
22. Phosphatidylserine in the treatment of clinically diagnosed Alzheimer’s disease. The SMID Group. J Neural Transm Suppl 1987;24:287–292.
23. Casamenti F, Scali C, Pepeu G: Phosphatidylserine reverses the age-dependent decrease in cortical acetylcholine release: a microdialysis study. Eur J Pharmacol 1991;194:11–16.
24. Calderini G, Aporti F, Bellini F, Bonetti AC, Teolato S, Zanotti A, Toffano G: Pharmacological effect of phosphatidylserine on age-dependent memory dysfunction. Ann NY Acad Sci 1985;444:504–506.
25. Engel Jr, Henry TR, Risinger MW, Sutherland WW, Chugani HT: PET in relation to intracranial electrode evaluations. Epilepsy Res Suppl 1992;5:111–120.