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Research Paper

Efficacy and Blood Plasmalogen Changes by Oral Administration of Plasmalogen in Patients with Mild Alzheimer's Disease and Mild Cognitive Impairment: A Multicenter, Randomized, Double-blind, Placebo-controlled Trial

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ABSTRACT

Background: Plasmalogens (Pls) reportedly decreased in postmortem brain and in the blood of patients with Alzheimer's disease (AD). Recently we showed that intraperitoneal administration of Pls improved cognitive function in experimental animals. In the present trial, we tested the efficacy of oral administration of scallop-derived purified Pls with respect to cognitive function and blood Pls changes in patients with mild AD and mild cognitive impairment (MCI).

Methods: The study was a multicenter, randomized, double-blind, placebo-controlled trial of 24 weeks. Participants were 328 patients aged 60 to 85 years who had 20 to 27 points in Mini Mental State Examination-Japanese (MMSE-J) score and five or less points in Geriatric Depression Scale-Short Version-Japanese (GDS-S-J). They were randomized to receive either 1 mg/day of Pls purified from scallop or placebo. The patients and study physicians were masked to the assignment. The primary outcome was MMSE-J. The secondary outcomes included Wechsler Memory Scale-Revised (WMS-R), GDS-S-J and concentration of phosphatidyl ethanolamine plasmalogens (PlsPE) in erythrocyte membrane and plasma. This trial is registered with the University Hospital Medical Information Network, number UMIN000014945.

Findings: Of 328 patients enrolled, 276 patients completed the trial (140 in the treatment group and 136 in the placebo group). In an intention-to-treat analysis including both mild AD ($20 \le$ MMSE-J ≤ 23) and MCI ($24 \le$ MMSE-J ≤ 27), no significant difference was shown between the treatment and placebo groups in the primary and secondary outcomes, with no severe adverse events in either group. In mild AD patients, WMS-R improved significantly in the treatment group, and the between group difference was nearly significant ($P = 0.067$). In a subgroup analysis of mild AD patients, WMS-R significantly improved among females and those aged below 77 years in the treatment group, and the between-group differences were statistically significant in females ($P = 0.017$) and in those aged below 77 years ($P = 0.029$). Patients with mild AD showed a significantly greater decrease in plasma PlsPE in the placebo group than in the treatment group.

Interpretation: Oral administration of scallop-derived purified Pls may improve cognitive functions of mild AD.

Funding: The Japanese Plasmalogen Society.

1. Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that has increased in prevalence as people live longer. It is estimated that the prevalence of AD may reach >74 million worldwide by 2030 (World Alzheimer Report, 2015). The cause and mechanism of AD is not fully elucidated, while progressive deposition of amyloid-β and Tau protein is considered to be a neuropathological hallmark of AD. On the other hand, the close connection between plasmalogens (Pls) and AD has been indicated by the observations of decreased phosphatidyl ethanolamine Pls (PlsPE) in the affected brain regions of AD patients, such as the hippocampus and frontal cortex (Ginsberg et al., 1995; Guan et al., 1999; Han et al., 2001). Decreased levels of PlsPE in the blood and

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cerebrospinal fluid of AD patients have been reported (Goodenowe et al., 2007; Wood et al., 2010, 2015; Oma et al., 2012; Yamashita et al., 2015). However, it is not clear whether the decrease of Pls in brain tissue and in plasma is the cause of the disease or merely a result of the disease. Recent studies of animal models of AD by our group indicated that intraperitoneal administration of purified Pls improved cognitive function (Katafuchi et al., 2012; Hossain et al., 2013, 2016).

Pls are a special class of glycerophospholipids characterized by a vinyl ether bond at the sn-1 position of glycerol backbone, and they are sometimes called plasmenyl phospholipid or alkenyl acryl phospholipid. They are found in almost all mammalian tissues and constitute about 18–20% of the total phospholipids in cell membranes. Predominant Pls in mammalian tissues are PlsPE and choline plasmalogen. PlsPE is much more abundant than choline plasmalogen except in heart and skeletal muscle. It is reported that Pls are abundant in the brain, retina, leukocytes (immune cells), sperm, heart, and skeletal muscle in mammals. This characteristic distribution of Pls indicates the importance of Pls in mammals (Farooqui and Horrocks, 2001; Braverman and Moser, 2012).

Pls are not only a structural component of animal cell membranes and reservoir for secondary messengers, but may also be involved in membrane fusion, ion transport, and cholesterol efflux, and act as antioxidants in cell membranes. Recently, the inhibitory effects of Pls on γ-secretase activity have been reported (Onodera et al., 2015). This study aimed to assess whether oral administration of Pls extracted from scallops would improve the cognitive function in patients with mild AD and mild cognitive impairment (MCI).

2. Methods

2.1. Study Design and Participants

This study was a multicenter, randomized, double-blind, placebo-controlled trial to evaluate the memory-improving efficacy of scallop-derived Pls in patients with mild AD and MCI. The study period consisted of a 24-week administration period and a four-week post-treatment period without administration (28 weeks in total).

Patients were those who had 20 to 27 points of Mini Mental State Examination Japanese Version (MMSE-J) scores, i.e., patients with mild AD ($20 \le$ MMSE-J ≤ 23) or those with MCI ($24 \le$ MMSE-J ≤ 27) (Solfrizzi et al., 2004). All patients were required to meet the criteria for mild AD or MCI set out in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V). Patients were confirmed to have no arteriosclerotic dementia with MRI or CT scans performed within the previous six months. Patients were also ensured to have five or less points of Geriatric Depression Scale Short Version in Japanese (GDS-S-J) in order to exclude depressive pseudodementia. Patients taking anti-Alzheimer drugs had no change in the regimen during the previous three months. All caregivers were required to accompany patients to all visits throughout the study, and provided information on patients' daily life and health status. Patients were excluded if they were allergic to scallops, the raw materials for test substance. Written informed consent was obtained from either patients or their caregivers. The study protocol was approved by the Institutional Review Boards of Fukuoka University Hospital (Fukuoka), Nihonbashi Sakura Clinic (Tokyo), and BOOCS Clinic Fukuoka (Fukuoka). The study was implemented in compliance with the Declaration of Helsinki.

2.2. Randomization and Masking

A computer-generated random allocation list was created by an expert at CAC Croit Corporation (Tokyo) based on the blocked randomization method with each block consisting of two placebo allocations and two Pls allocations. Each study site was provided with test substance kits numbered according to the allocation list, which were handed directly to patients. At some study sites without cold storage space, patients received test kits by courier every month. Thus enrolled patients were randomly assigned to receive either 1.0 mg Pls/day or placebo. Test substance was jelly-like substance. Active substance and placebo were identical in appearance and taste. Patients, caregivers, study physicians, and clinical staff were masked to treatment allocation throughout the study period.

2.3. Procedures

Enrolled patients were provided with the test substance at baseline visit or within one week after the baseline visit, and were instructed to take it orally twice/day for 24 weeks. They received one month's extra test substance in case they missed the next scheduled visit. To confirm compliance, they were requested to return unconsumed test substance at their next visit. They were furthermore urged not to change the regimen during the study period as far as possible. If a patient changed his or her drug use, we terminated his or her observation at the point. We recorded any complications and adverse events reported by patients at each visit.

The primary outcome measure was MMSE-J. The secondary outcome measures included Wechsler Memory Scale-Revised (WMS-R), GDS-S-J, plasma PlsPE levels, and relative concentration of PlsPE in erythrocyte membrane, namely the percentage of Pls to the total phospholipids in erythrocyte membrane. Cognitive function was assessed at baseline, and at weeks 12, 24, and 28. Blood was drawn from patients in the fasting state at baseline, and at weeks 8, 16, 20, 24, and 28 for measuring erythrocyte PlsPE and plasma PlsPE. PlsPE measurement was performed using the previously reported method (Mawatari et al., 2007, 2016).

Safety assessment was conducted by recording adverse events and performing a physical examination and biochemical blood tests such as liver function, renal function, blood sugar, and lipid levels at each visit.

2.4. Statistical Analysis

To determine the sample size, we assumed that the MMSE-J score would improve by 5% in the placebo group and by 10% in the Pls treatment with SD of 15% in each. With a statistical power of 0.80 and a one-sided significance level of 0.01, the required sample size was estimated to be 181 in each group. The target number of enrollments was decided to be 200 in each with allowance for some extent of dropout.

The between-treatment difference was assessed by unpaired *t*-test, and the within-group change from the baseline was evaluated by paired *t*-test. In terms of after-treatment outcome of the cognitive function, the mean change from the baseline and 95% confidence interval (CI) were presented. Analyses were done with Stata version 13 (StataCorp, College Station, TX). The trial is registered at the University Hospital Medical Information Network as ID UMIN000014945.

2.5. Role of the Funding Source

B&S Corporation Co. Ltd. (Tokyo), one of the donors to The Japanese Plasmalogen Society, was involved in provision of Pls test substance and placebo and delivery to study sites, but it had no involvement in study design and planning, investigator training, data analysis, interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. All co-investigators also had full access to the data.

3. Results

3.1. Study Participants

A total of 328 patients were enrolled at 25 hospitals or clinics in Kyushu, Kanto, and Kansai regions in Japan from November 15, 2014

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