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Original article

DL−/PO−phosphatidylcholine serves as a memory enhancer for normal healthy subjects

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ABSTRACT

Purpose: The present study investigated the effect of 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DL−PC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PO−PC) on learning and memory in normal healthy mice and humans.
Study section and results: In the water maze test, normal mice orally co-administered DL−PC (1 mg/kg) and PO−PC (1 mg/kg) showed significantly shortened retention latency compared to mice administered polyethylene glycol; however, the acquisition latency was not affected. For humans, a memory test was carried out on 34 subjects; they had 5 min to learn 3 different kinds of 2 Chinese compound words, a Japanese poem consisting of 17 syllables, and a sentence chosen from an old Japanese essay. One minute (short-term memory test) and 1 week (long-term memory test) later, they wrote what they had learned. For subjects taking DL−PC (50 mg/day) and PO−PC (45 mg/day), the percentage of correct answers for both the short- and long-term memory tests increased in a trial-number-dependent manner. The percentage of correct answers in the long-term memory test at the third trial for subjects taking DL−/PO−PC was nearly 3 times higher than that of control subjects, even though there was no significant difference in the percentage of correct answers in the short-term memory test between the 2 groups.
Conclusion: The results of the present study indicate that DL−/PO−PC could enhance long-term memory potential for normal healthy subjects.
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1. Introduction

Phospholipids such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol are implicated in the regulation of a wide variety of bioreactions. In our previous study, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine (DL−PC) potentiated α7 nicotinic acetylcholine (nACh) receptor responses and facilitated hippocampal synaptic transmission in an α7 nACh receptor-dependent manner [1]. The α7 nACh receptor plays a critical role in the expression of long-term potentiation (LTP), a cellular model of learning and memory [2−9]. DL−PC, therefore, could improve learning impairments by targeting the α7 nACh receptor. In contrast, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (PO−PC) enhanced long-term depression (LTD), another cellular model of learning and memory, in concert with decreased expression of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit GluR1 on the plasma membrane [10]. Consequently, PO−PC may also improve memory disorders. Indeed, DL− and/or PO−PC ameliorates cognitive decline in humans [10,11]. Interestingly, taking a combination of DL− and PO−PC had an additive effect that was more beneficial in treating cognitive impairment than was the intake of either DL−PC or PO−PC alone [11].

Alzheimer disease is characterized by severe dementia in association with very rapidly progressive brain atrophy. Although no promising drug has been identified, ACh esterase inhibitors (AChEIs), such as donepezil, galantamine, and rivastigmine, have been clinically used in Alzheimer disease to alleviate symptoms [12]. We recently found that DL−/PO−PC has the potential to ameliorate Alzheimer dementia, and is more beneficial with a higher efficacy than donepezil (unpublished data). Thus, DL−/PO−PC could be useful for the treatment of cognitive decline and

Abbreviations: DL−PC, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine; PO−PC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; ACh, Acetylcholine; nACh, nicotinic ACh; LTP, long-term potentiation; LTD, long-term depression; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; AChEIs, acetylcholinesterase inhibitors; PEG, polyethylene glycol; PLSD, Protected Least Significant Difference.

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dementia. The present study was conducted to understand the effect of DL−/PO−PC on learning and memory abilities of normal, healthy subjects.

To address this question, we carried out a water maze test by using normal mice and a memory test that we devised for healthy humans. We show here that DL−/PO−PC serves as a memory enhancer for normal healthy subjects.

2. Materials and methods

2.1. Animal care

All procedures were approved by the Animal Care and Use Committee at Hyogo College of Medicine and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Participants

The study with human subjects was approved by the Institutional Review Board at Hyogo College of Medicine, and informed consent was obtained from all the participants.

2.3. Water maze test

A circular plastic water tank 100 cm in diameter and 35 cm deep was used for the water maze test. The entire inside of the pool was painted white, and the pool was filled to a depth of 25 cm with muddy water containing India ink at 22 °C. A platform (11 cm in diameter) painted white was placed into the pool, such that the top was 1 cm below the water surface. The pool was put in a test room containing several marks that mice were able to see from the pool. The position of the marks remained unchanged throughout testing. A platform was located in a constant position, i.e., in the middle of one quadrant, equidistant from the center and edge of the pool. Male C57BL/6 mice (7 weeks old) facing the wall of the pool were placed into water at 1 of 5 positions selected at random, and the time from start to escape onto the platform (acquisition latency) was measured. DL− and PO−PC dissolved in polyethylene glycol (PEG) (final volume, 0.1 ml) or PEG alone (final volume, 0.1 ml) were orally administered to mice for 16 days from the beginning to the end of all phases of the water maze test. There were 2 trials of the water maze task per day, and the second trial began 1 min after the end of the first trial. Mice participated in the task for 8 consecutive days and the acquisition latency (time from start to arrival onto the platform) was measured.

Eight days later, the platform was removed and the retention latency (time from start to arrival to the place where the platform had been set, 90 s maximum) was measured.

2.4. Memory test

The memory test that we devised was carried out in 34 healthy humans, who were divided into 2 groups, i.e., 20 subjects aged 24–56 years (average, 38 ± 2 years; male:female = 6:14) took both DL−PC (50 mg/day) and PO−PC (45 mg/day) orally everyday for 1 month prior to the test (DL−/PO−PC-intake group), and 14 subjects aged 23–49 years (average, 35 ± 3 years; male:female = 2:12) took nothing (control group). All subjects had 5 min to learn 3 different kinds of 2 Chinese compound words, a Japanese poem consisting of 17 syllables, and a sentence chosen from an old Japanese essay, as shown in Fig. 2. They were then asked to recall the words, the poem, and the sentence that they remembered 1 min (short-term memory test) and 1 week (long-term memory test) after the test, and the percentage of correct answers was calculated.

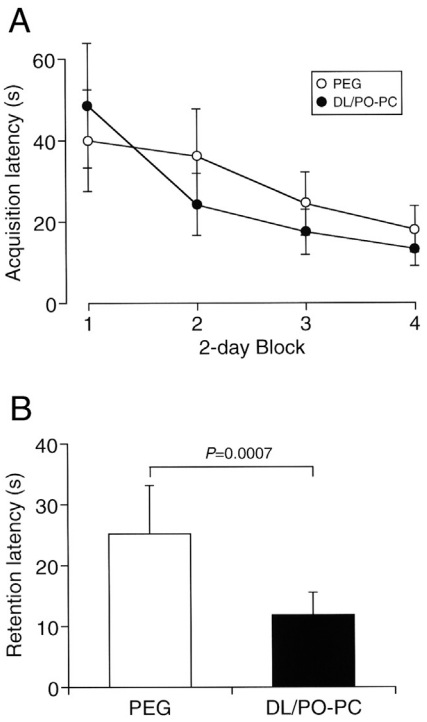

Fig. 1. Effect of DL−/PO−PC on spatial learning and memory. Mice were orally administered DL−PC (1 mg/kg) plus PO−PC (1 mg/kg) (DL−/PO−PC) or PEG everyday from the beginning to the end of all phases of the water maze test. (A) Each point represents the mean (±SEM) acquisition latency from 2 consecutive days ($n = 10$). (B) Each column represents the mean (±SEM) retention latency ($n = 10$). P value, unpaired t-test.

2.5. Statistical analysis

Statistical analysis was carried out using Fisher's Protected Least Significant Difference (PLSD) test and unpaired t-test.

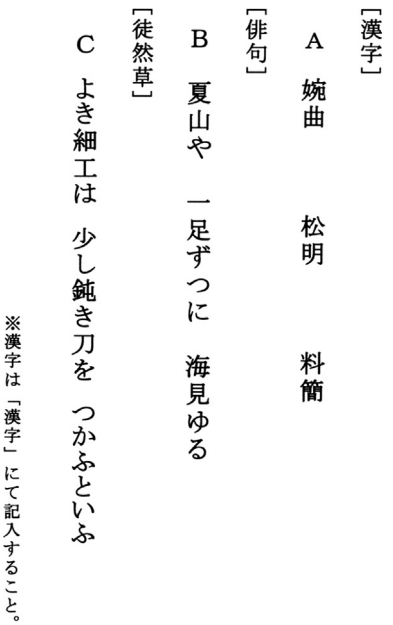

Fig. 2. An example of the memory test including 2 Chinese compound words, a Japanese poem consisting of 17 syllables, and a sentence chosen from an old Japanese essay.

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3. Results and discussion

The water maze test was carried out in mice with oral administration of DL–PC (1 mg/kg) and PO–PC (1 mg/kg) (DL–/PO–PC group) or PEG (PEG group). There was no significant difference in the swimming speed between the PEG (63.0 ± 13.4 mm/s) and DL–/PO–PC groups (82.9 ± 20.5 mm/s) ($P = 0.0550$, unpaired t-test), indicating that DL–/PO–PC has no effect on swimming speed.

Oral co-administration of DL–PC (1 mg/kg) and PO–PC (1 mg/kg) did not affect the acquisition latency compared to the latency from the PEG group ($P = 0.1651$, Fisher's PLSD test) (Fig. 1A). In contrast, the retention latency for the DL–/PO–PC group was significantly shorter than the latency for the PEG group (Fig. 1B). Taken together, these results indicate that DL–/PO–PC is capable of enhancing memory ability rather than learning ability for normal mice.

We next carried out a memory test on normal healthy humans who were untreated (control group) or were taking DL–PC (50 mg/day) and PO–PC (45 mg/day) orally (DL–/PO–PC-intake group). For the DL–/PO–PC-intake group, the percentage of correct answers on both the short- and long-term memory tests increased in a trial-number-dependent manner, but this effect was not found with the control group (Fig. 3A,B). The percentage of correct answers in the long-term memory test at the third trial was significantly higher for the DL–/PO–PC-intake group than for the control group, with the DL–/PO–PC-intake group having approximately 3 times as many correct answers (Fig. 3C). In contrast, there was no significant difference in the percentage of correct answer in the short-term memory test between the 2 groups (Fig. 3C). This indicates that DL–/PO–PC has the potential to enhance long-term memory ability in healthy humans.

DL–PC induces LTP by targeting α7 nACh receptor [1], suggesting that DL–PC is a learning enhancer. In contrast, PO–PC by itself potentiates LTD by decreasing cell surface localization of the GluR1 AMPA receptor [10], suggesting that PO–PC may be a memory enhancer. We have provided evidence that DL– and/or PO–PC actually ameliorates cognitive decline and dementia in humans [10,11]. The results of the present study clearly demonstrate that DL–/PO–PC serves as a memory enhancer even in normal, healthy subjects. Thus, DL–/PO–PC may not only avert a variety of dementias including Alzheimer disease in humans, but also enhance cognitive functions. DL–/PO–PC has the advantage that it is a natural substance in living cells and exhibits few side effects. Therefore, many humans could safely and easily take DL–/PO–PC for improving brain health.

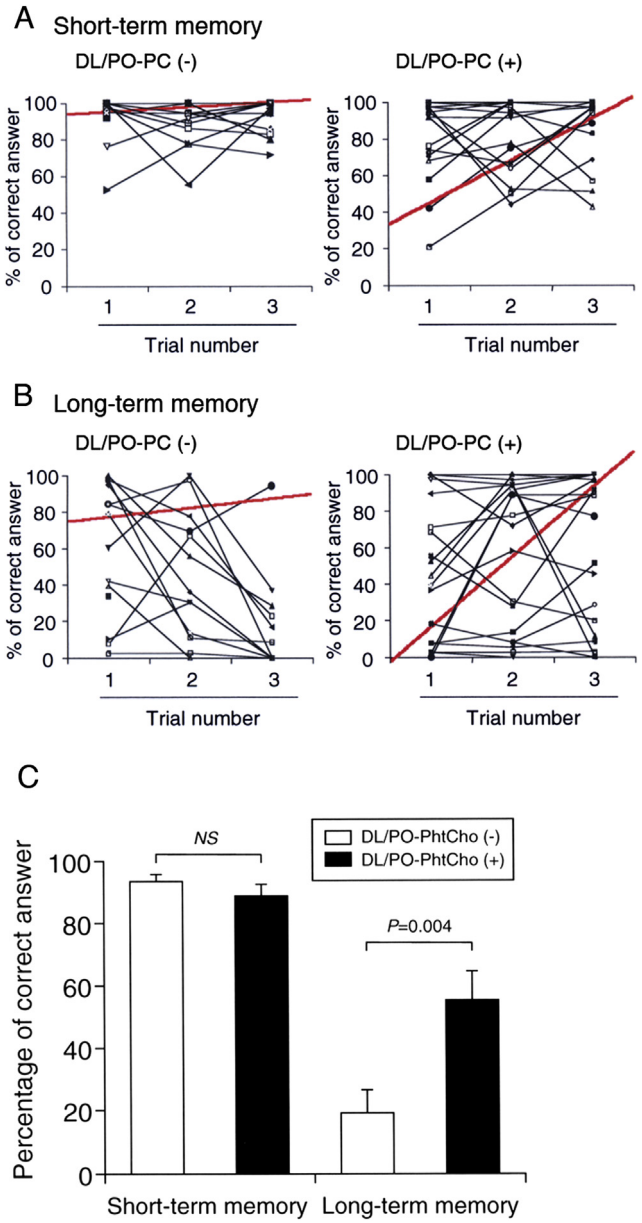

Fig. 3. Effect of DL–/PO–PC on memory in normal, healthy humans. A memory test was carried out on 14 untreated subjects [DL–/PO–PC (–)] and 20 subjects with taking both DL–PC (50 mg/day) and PO–PC (45 mg/day) orally [DL–/PO–PC (+)]. (A) The percentages of correct answers on the short-term memory test (1 min after the trial) at each trial for individual subjects from the control group [DL–/PO–PC (–)] and the group taking DL– and PO–PC [DL/PO–PC (+)] were plotted and lines fitted to each point were drawn. (B) The percentages of correct answer for the long-term memory test (1 week after the trial) at each trial for individual subjects from the control group [DL–/PO–PC (–)] and the group taking DL– and PO–PC [DL–/PO–PC (+)] were plotted and the lines fitted to each point were drawn. (C) Each column represents the mean (±SEM) percentage of correct answers in the short- and long-term memory tests at the third trial for untreated subjects [DL–/PO–PC (–)] and subjects taking DL– and PO–PC [DL–/PO–PC (+)]. P value, unpaired t-test. *NS*, not significant.

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