Effects of Oral Administration of Soybean Lecithin Transphosphatidylated Phosphatidylserine on Impaired Learning of Passive Avoidance in Mice

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ABSTRACT—Soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS) was investigated for its effect on the impaired learning of a passive avoidance task by mice induced by scopolamine or cycloheximide. SB-tPS (240, 360, 480 mg/kg) administered orally significantly prolonged the step-through latency shortened by scopolamine. SB-tPS (240 mg/kg) administered orally also prolonged the step-through latency shortened by cycloheximide. These results suggest that the effect of SB-tPS on the impaired learning behavior may be related not only to the cholinergic system but also the serotonergic system.

Keywords: Phosphatidylserine, Passive avoidance, Scopolamine

After Toffano and Bruni reported in 1980 that brain cortex phosphatidylserine (B-PS), a pharmacologically active phospholipid, counteracts age-related changes in the central nervous system (CNS) (1), many investigators have observed that exogenous B-PS affects neurotransmission (2-4) and behavioral performance (5, 6) by modifying the composition and function of the neuronal membrane, which is associated with aging (7). Clinical investigations have also shown that B-PS treatment can improve Alzheimer’s disease (AD) and related cognitive disorders (8, 9). Sakai et al. (10) prepared soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS) from soybean phosphatidylcholine by transphosphatidylation using phospholipase D, and they found that the intravenous or intraperitoneal administration of SB-tPS increases brain glucose concentrations in mice and antagonizes amnesic effects of scopolamine (SCP) in rats, as did B-PS.

In the present study, we investigated the effect of the oral administration of SB-tPS on the SCP- or cycloheximide (CYH)-induced impairment of passive avoidance performance in mice, which is a useful model for the evaluation of a drug’s effects and for investigations of mechanisms of learning behavior enhancers. We also investigated the effect of oral SB-tPS on the pain threshold of mice.

For the passive avoidance task, five-week-old male mice of the ddY strain (Shizuoka Laboratory Animal Center, Shizuoka), weighing 26–32 g, were used.

A step-through type apparatus (Muromachi Kikai, Tokyo) was used for the passive avoidance task. The apparatus consisted of two compartments, an illuminated compartment (7.5 x 9.3 x 15 cm, with a 60 W light suspended 20 cm above the top of chamber) and a dark compartment (12.5 x 14 x 16.5 cm). The compartments were separated by a guillotine door (3 x 3 cm). In the acquisition trial, the mouse was gently placed in the small lighted chamber facing away from the guillotine door. The door was raised, and as soon as mouse entered the dark chamber, an electrical foot shock (1 or 2 mA, shock scrambler SGS-001; Muromachi Kikai) was applied to the floor grid until the mouse ran back into the lighted chamber. The mouse could escape from the shock only by returning to the illuminated compartment. Thereafter, the mouse was returned to its home cage.

SCP (0.5 mg/kg, i.p.) or CYH (150 mg/kg, s.c.) was administered 30 min before the acquisition trial. SB-tPS (60, 120, 240, 360 or 480 mg/kg, p.o.) and tetrahydroaminoacridine (THA, 5 mg/kg, i.p.), an acetylcholinesterase inhibitor, were administered to all mice 60 and 20 min before the acquisition trial, respectively. Eight to twelve mice were used per group. About 24 hr after the acquisition trial, the retention trial was conducted by placing the mouse in the illuminated chamber and...
measuring the step-through latency (LT). The maximum observation period in this test was 300 sec.

The effect of SB-tPS on the pain threshold of ddY mice was studied by the hot plate method. Using a thermister, the plate was maintained at a temperature of 55°C to 56°C. The pain threshold was evaluated by measuring the appearance time of behavior such as jumping, licking or lifting of the paws. The maximum heat exposure was set at 45 sec to protect the mice from burning their paws. SB-tPS (240 or 480 mg/kg) was administered orally 60 min before the hot plate test to 7 mice. As control mice, distilled water (D.W.) was administered orally to 6 mice.

SB-tPS was prepared from soybean phosphatidylcholine (PC80™; Croklaan b.v., Wormerveer, Holland) as reported in a previous paper (10). SCP, CYH and THA were obtained from Sigma Chemicals (St. Louis, MO, USA).

SB-tPS was suspended in distilled water and sonicated for 30 min and administered orally in a volume of 0.1 ml per 10 g mouse body weight. SCP, CYH and THA were each dissolved in saline and administered in a volume of 0.1 ml per 10 g mouse body weight.

Statistical analyses were performed by analysis of variance (ANOVA) and the Wilcoxon rank-sum test for non-parametric systems. The significance of differences in the step-through latency of the passive avoidance response among groups was examined by the Kruskal-Wallis test, followed by the Dunnett multiple comparison test.

The effect of SB-tPS on SCP-induced amnesia in passive avoidance learning was investigated.

The mean LT to enter the dark compartment in the acquisition trial was not different among the groups. In the retention trial, the LT of the control mice, which were given saline 30 min after the administration of D.W. alone, was 286.3 ± 11.0 (mean ± S.E. sec). The administration of SCP (0.5 mg/kg, i.p.) 30 min after the administration of D.W. significantly shortened the LT (62.8 ± 14.9 sec, P < 0.01). Given at 10 min after the SCP, the administration of THA (5 mg/kg, i.p.) prolonged the LT shortened by SCP. SB-tPS (60 or 120 mg/kg), administered 60 min before the acquisition trial, did not have a significant effect, but at the dose of 240 mg/kg, it prolonged the shortened LT induced by SCP (Fig. 1A). In another experiment, SB-tPS (240, 360 and 480 mg/kg) also significantly prolonged the LT shortened by SCP (Fig. 1B). The dose-response curve was bell-shaped.

CYH (150 mg/kg, s.c.) significantly shortened the LT. SB-tPS (240 or 480 mg/kg, p.o.) administered 60 min be-

Fig. 1. Effects of soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS) and tetrahydroaminoacridine (THA) on the scopolamine (SCP)-induced impairment of the passive avoidance response in mice. A: SB-tPS (60, 120 or 240 mg/kg) and THA. B: SB-tPS (240, 360 or 480 mg/kg). SCP (0.5 mg/kg, i.p.) was administered 30 min before the acquisition trial. SB-tPS and THA (5 mg/kg, i.p.) were administered 60 and 20 min before the acquisition trial, respectively. Ordinate indicates latency time (LT) to enter the dark compartment. Each column indicates the mean ± S.E. **P < 0.01: significantly different from the value for control mice, #P < 0.05, ##P < 0.01: significantly different from the SCP group (VEH). Numbers of mice are given at the center of columns.
fore the acquisition trial significantly prolonged the LT shortened by CYH (Fig. 2: A and B).

The administration of SB-tPS (240 or 480 mg/kg, p.o.) did not change the pain threshold of the mice in the hot plate test (Table 1).

Many cholinomimetics have been synthesized recently, and they are now under clinical study for the treatment of senile dementia and Alzheimer's disease, based on the findings that cholinergic function plays an important role in learning and memory (12). In the present study, the oral administration of SB-tPS synthesized from soybean lecithin significantly attenuated the SCP-induced amnesia in mice. SB-tPS did not affect the pain response or the mean LT in the acquisition trial at doses that ameliorated the impaired learning behavior. The actions of SB-tPS may thus be due to a facilitation of the cognitive function. Although there is as yet little direct evidence that SB-tPS involves central cholinergic mechanisms, these results suggest that the anti-amnesia effect of SB-tPS could be attributed to the activation of cholinergic neuronal systems in the CNS. Indeed, regarding mechanism of B-PS, several investigators have proposed a cholinergic mechanism based on the findings that the acute administration of B-PS antagonizes the amnesic effects of SCP on spontaneous alternation and passive avoidance (5, 6).

CYH, a protein synthesis inhibitor, interferes with

![Fig. 2. Effects of soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS) on the cycloheximide (CYH)-induced impairment of the passive avoidance response in mice. A: SB-tPS (120 or 240 mg/kg). B: SB-tPS (240, 360 or 480 mg/kg). CYH (150 mg/kg, s.c.) was administered 30 min before the acquisition trial. SB-tPS was administered orally 60 min before the acquisition trial. Ordinate indicates latency time (LT) to enter the dark compartment. Each column indicates the mean±S.E. **P<0.01: significantly different from the value for control mice. *P<0.05: significantly different from the CYH group (VEH). Numbers of mice are given at the center of columns.]

### Table 1. The effect of SB-tPS on the pain threshold of ddY mice

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose (mg/kg, p.o.)</th>
<th>N</th>
<th>Pain threshold: incidence-response time (sec) before</th>
<th>20 min</th>
<th>40 min</th>
<th>60 min</th>
<th>120 min</th>
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<tr>
<td>D.W.</td>
<td></td>
<td>6</td>
<td>6.5±1.2</td>
<td>9.8±2.3</td>
<td>9.5±1.6</td>
<td>11.9±3.0</td>
<td>11.8±1.3</td>
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<tr>
<td></td>
<td></td>
<td>7</td>
<td>6.3±0.9</td>
<td>11.0±1.3</td>
<td>9.9±1.0</td>
<td>9.1±0.7</td>
<td>12.7±2.1</td>
</tr>
<tr>
<td>SB-tPS</td>
<td>240</td>
<td>7</td>
<td>7.4±1.3</td>
<td>9.7±1.6</td>
<td>10.1±2.5</td>
<td>12.2±2.2</td>
<td>11.2±1.5</td>
</tr>
<tr>
<td></td>
<td>480</td>
<td>7</td>
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SB-tPS was administered to mice 60 min before the hot plate test. Each value is expressed as the mean±S.E. for 6–7 mice.
learning behavior by disturbing various neurotransmitter systems, including the central monoaminergic (13) and cholinergic systems (14). In addition, it has recently been proposed that the place-navigation deficits seen in some aged rats may reflect a combined cholinergic-serotonergic impairment (15). We therefore investigated the ameliorating effects of SB-tPS on the impaired learning behavior induced by CYH. We found that SB-tPS also had an ameliorating effect on the impairment of passive avoidance learning behavior caused by CYH. These results suggest that the effect of SB-tPS on the impaired learning behavior may be related not only to the cholinergic system but also the serotonergic system.

In the treatment of senile dementia of the Alzheimer type and of multi-infarct dementia, patients require a long period of drug treatment. From this point of view, it is better for drugs to be administered orally and preferable that they have a long active-period in the body. Further investigation will be directed at demonstrating the existence of SB-tPS in the brain after oral administration, since the question arises as to whether exogenous SB-tPS penetrates into the brain and fuses with membranes.

In the present study, the oral administration of SB-tPS had an ameliorating effect on SCP or CYH-induced amnesia in mice, and this effect may be related not only to central cholinergic but also serotonergic neuronal systems.

REFERENCES