Oral Administration of Soybean Lecithin Transphosphatidylated Phosphatidylserine (SB-tPS) Reduces Ischemic Damage in the Gerbil Hippocampus

Satoru Suzuki, Masayoshi Furushiro, Masatoshi Takahashi, Masashi Sakai* and Satoshi Kudo
Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 185–8550, Japan
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ABSTRACT—Mongolian gerbils orally administered with soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS, 240 mg/kg) for 5 days were subjected to cerebral ischemia by bilateral common carotid artery occlusion. The pyramidal cell damage of the hippocampal CA1 subfield was classified into 4 grades according to the proportion of damaged neurons on the tenth day after the ischemic treatment. The damage score of the SB-tPS group was statistically less than that of the control group. This suggests that the pre-administration of SB-tPS may relieve the delayed neuronal cell death caused by cerebral ischemia.

Keywords: Phosphatidylserine, Oral administration, Cerebral ischemia

In 1986, it was first reported that oral administration of bovine cortex-derived phosphatidylserine (BC-PS) to senile dementia patients improved their cognitive disorders (1). Since then, the effectiveness of BC-PS has been confirmed by several double-blind, placebo-controlled studies (2, 3). Recently, soybean lecithin transphosphatidylated phosphatidylserine (SB-tPS), which was enzymatically prepared from soybean lecithins and L-serine by a phospholipase D reaction, was also shown to recover the scopolamine-induced amnesia in rodents (4, 5) as BC-PS did, although the fatty acid composition was different from that of BC-PS.

While the effects of phosphatidylserines on the memory function have been well documented, there is no report describing the effect of phosphatidylserine on cerebral ischemia that irreversibly damages neurons and is considered to be one of the major causes of senile dementia.

In this report, the preventive effect of SB-tPS oral administration on the ischemic damage of hippocampal CA1 subfield pyramidal cells, which are important for memory and more sensitive to ischemic treatment than other neurons (6), was examined.

Fourteen-week-old male Mongolian gerbils (Japan SLC, Hamamatsu) were maintained under controlled temperature (24±2°C), humidity (55±10%) and lighting (8:30–20:30). The animals were divided into groups of 4 or 5 per cage and fed MF diet (Oriental Kobo, Tokyo) and water ad libitum.

SB-tPS was prepared from soybean phosphatidylcholine (PC80™; Croklaan b.v., Wormerveer, Holland) and L-serine by the previous method (5) with a slight modification. SB-tPS containing 95% phosphatidylserine was emulsified with distilled water by sonication and orally administered to 17 gerbils (240 mg/kg) once a day for 5 days before an ischemic treatment. Distilled water was given to 18 gerbils as the control.

On the day of operation, the gerbils were anesthetized with 2% halothane and placed in a supine position. A midline incision was made in the ventral neck, and the carotid arteries were carefully separated from the adjacent vein sympathetic nerves. After reducing the halothane concentration to 1%, the carotid arteries were clamped with Sugita aneurysm clips (Mizuho Ikakogyo, Tokyo). Fifteen minutes later, the clips were removed to allow blood reperfusion. No difference in behavior and body weight changes between those two groups was observed after the ischemic treatment. As a sham-operation, similar manipulation without clamping was done to 3 gerbils.

On the tenth day after the ischemic treatment, the animals were anesthetized with diethyl ether and perfused with 2% paraformaldehyde and 2.5% glutaraldehyde through the heart. The brain was removed from the skull, kept in 10% formaldehyde, and then embedded in
paraffin. Coronal sections of 5-μm thickness were taken 1.5- and 2.0-mm posterior to the bregma, and then they were stained by the hematoxylin-eosin method or the Nissl method.

The specimens of the both hippocampal sides were observed by microscopy and the necrotic pyramidal cells of the CA1 subfield were counted and classified into 4 scores according to the proportion of the necrotic cells: 0–10% [score 1], 10–50% [score 2], 50–90% [score 3], 90–100% [score 4] (Fig. 1, A–D).

One gerbil in the SB-tPS group and 4 in the control group died within 10 days after the operation. In these animals, necrosis and/or loss of pyramidal cells were found in most of the hippocampal area and infiltration of neutrophils into the hippocampus or enlargement of the lateral ventricle was also observed. In addition, in both the SB-tPS and the control groups, 4 specimens in each were excluded due to failure of ischemic treatment or specimen preparation. Consequently, 28 specimens (both sides of the hippocampus) from the SB-tPS group and 24 specimens from the control group were pathologically examined.

As shown in Fig. 2, of the 24 specimens in the control group, 19 (79%) were classified as score 4, the most severe damage, while 19 (68%) in the SB-tPS group were classified as score 3 and number with score 4 was only 6 (21%). The distributions of the scores between those two groups were significantly different ($P < 0.0001$, $\chi^2$ analysis). All specimens of the sham-operated animals were classified as score 1.

This is the first report that the pre-administration of phosphatidylserine alleviates the delayed neuronal cell death of the hippocampal CA1 subfield by ischemia.

Although the mechanisms are unclear, the following explanations are possible. Firstly, energy exhaustion in brain caused by ischemia (7) may be compensated with

**Fig. 1.** Representative microphotographs of hippocampal CA1 subfield in gerbils 10 days after the operation. A: score 1 (the proportion of the damaged neurons is 0–10%, a sham-operated animal). B: score 2 (10–50%, a control animal). C: score 3 (50–90%, a SB-tPS-administered animal). D: score 4 (90–100%, a control animal). Hematoxylin-eosin staining. Magnification: $\times 180$. 
the supply of glucose augmented by the SB-tPS administration (5), which may reduce subsequent abnormal release of glutamate or intracellular acidosis. Another possibility is that SB-tPS, through its antioxidant effect (8, 9), may reduce the cell membrane damage due to lipid peroxidation induced by radicals during the blood reperfusion after the ischemic treatment. SB-tPS may also prevent ischemic damage through suppressing cytotoxic factors like tumor necrosis factor-α (TNF-α) or excessive nitric oxide (NO), because phosphatidylserine has been shown to reduce the release of TNF-α (10) or the activity of NO synthase (11). Finally, an intravenous administration of BC-PS was reported to lower the rectal temperature of rats from 39°C to 37.5°C (12). Lowering the body temperature is known to enhance the resistance to ischemic damage (13). So this action may contribute to an anti-ischemic mechanism of SB-tPS, though the body temperature was not measured in this experiment.

An intracerebroventricular injection of phosphatidylserine to aged rats was reported to restore the memory impairment (14), suggesting the direct action of this phospholipid on the brain. However, whether SB-tPS acts directly on brain cells or through its metabolites and/or humoral factors is a subject for further investigation.

In this study, a consecutive oral-administration of SB-tPS before an ischemic insult displayed a beneficial effect against the damage of gerbil hippocampal subfield CA1 pyramidal cells. Further work is required to determine if this finding can be extended to prophylactic use of SB-tPS for cerebrovascular dementia.

REFERENCES