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Protective Effect of Soybean-Derived Phosphatidylserine on the Trimethyltin-Induced Learning and Memory Deficits in Rats

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The present study examined the effects of soybean-derived phosphatidylserine (SB-PS) on the learning and memory function and the neural activity in rats with trimethyltin (TMT)-induced memory deficits. The cognitive improving efficacy of SB-PS on the amnesic rats, which was induced by TMT, was investigated by assessing the Morris water maze test and by performing cholineacetyl transferase (ChAT), acetylcholinesterase (AChE) and cAMP responsive element binding protein (CREB) immunohistochemistry. A positron emission tomography (PET) scanning the rat brain was by performed administer 18F-Fluorodeoxy-glucose (18F-FDG). The rats with TMT injection showed impaired learning and memory of the tasks and treatment with SB-PS produced a significant improvement of the escape latency to find the platform in the Morris water maze at the 2nd day compared to that of the MCT group. In the retention test, the SB-PS group showed increased time spent around the platform compared to that of the MCT group. Consistent with the behavioral data, SB-PS 50 group significantly alleviated the loss of acetyl cholinergic neurons in the hippocampus compared to that of the MCT group. Treatment with SB-PS significantly increased the CREB positive neurons in the hippocampus as compared to that of the MCT group. In addition, SB-PS groups increased the glucose uptake in the hippocampus and SB-PS 50 group increased the glucose uptake in the frontal lobe, as compared to that of the MCT group. These results suggest that SB-PS may be useful for improving the cognitive function via regulation of cholinergic marker enzyme activity and neural activity.

Key words : Trimethyltin (TMT), Cholineacetyl transferase (ChAT), Acetylcholinesterase (AChE), cAMP responsive element binding protein (CREB), soybean-derived phosphatidylserine (SB-PS)

Introduction

Alzheimer's disease (AD) is the most common form of age-related dementia in the elderly. The current number of people who suffer from AD is estimated to be 25 to 30 million worldwide. AD is a progressive neurodegenerative brain disorder that gradually destroys a patient's memory and ability to learn, make judgments, communicate with the social environment and carry out daily activities. In the course of the disease, short-term memory is affected first, caused by neuronal dysfunction and degeneration in the hippocampus and amygdala. As the disease progresses further, neurons also degenerate and die in other cortical regions of the brain[1]. At that stage, sufferers often experience dramatic changes in personality and behavior, such as anxiety, suspiciousness or agitation, as well as delusions or hallucinations[2]. AD patients show a progressive neuronal cell loss that is associated with region-specific brain atrophy. In particular, the cholinergic projection from the nucleus basalis of Meynert to areas of the cerebral cortex is the pathway that is very early and most severely affected in brains from AD patients[3]. Loss of basal forebrain cholinergic neurons is demonstrated by reductions in number of cholinergic markers such as cholineacetyl transferase (ChAT), muscarinic acetylcholine receptor (mAChR) and nicotinic acetylcholine receptor (nAChR) binding, as well as levels of acetylcholine (ACh) itself[4]. These changes are highly correlated with the degree of dementia in AD.

Phosphatidylserine (PS) is a member of the membrane phospholipids that is especially abundant in the brain. Because

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of its presence in the brain, effects of PS on the central nervous system have been widely investigated[5]. Several studies have shown that PS extracted from bovine cortex phosphatidylserine (BC-PS) improves the cognitive function of the elderly including AD patients and people with age associated memory impariment[6]. However, the use of BC-PS in medicine or dietary supplements is now discouraged. Because of the risk of bovine spongiform encephalopathy (BSE)[7] and only about 3 grams of PS can be obtained from one bovine cortex. PS which originated from plant tissues has been considered a possible alternative of BC-PS. Efforts to overcome these problems have led to the development of soybean-derived phosphatiedylserine (SB-PS). SB-PS is a phosphatidylserine made from soybean lecithin by enzymatic reaction with L-serine[8]. Several clinical studies have shown that the oral administration of soybean-originated PS to aged rats resulted in a significant improvement in memory and other cognitive functions[9,10] In addition, the intraperitoneal or oral administration of SB-PS to various rodents improved scopolamine-induced amnesia in passive avoidance tasks[11], and cognitive disorder in senile subjects[10].

Trimethyltin chloride (C3H9ClSn) (TMT) is a potent neurotoxicant that selectively induces neuronal death in both human and animal limbic system, and in particular in the hippocampal formation[12,13]. This substance is regarded as being particularly useful for studying the response to injury on account of the distinct pattern of degeneration it causes in rodent brain. In particular, the rat hippocampus constitutes the most suitable model for TMT-induced brain injury[14]. The molecular basis for the selective vulnerability of specific neuronal populations to neuronal insults has been a key focus in the fields of neurology and neuropathology[15]. TMT-induced neurodegeneration is characterized by massive neuronal death that is mainly localized in the limbic system and especially in the hippocampus, and this is accompanied by reactive gliosis, epilepsy and marked neurobehavioral alterations, and so this is considered a useful model of neurodegeneration and selective neuronal death[15].

Intoxication with TMT leads to profound behavioral and cognitive deficits in both humans and experimental animal[16].

In experimental animals TMT administration induces seizures, behavioral alterations (hyperactivity, tail mutilation, vocalization and hyper excitability and aggression) and cognitive deficits (memory loss and learning impairment) referred to severe hippocampal damage[17].

In rats, TMT induces the degeneration of pyramidal neurons in the hippocampus and the cortical areas (pyriform cortex, entorhinal cortex, subiculum) connected to the hippocampus, but there is also neuronal loss in the association areas[18]. Furthermore, behavioral studies have shown increased locomotor activity, disruption in self-grooming and learning deficits in TMT-intoxicated rats[12,13,19]. TMT intoxication impairs the performance of learning acquisition of water maze and Biel maze (water avoidance) tasks as well as the performance of Hebb-Williams maze and radial arm maze tasks[14,20,21]. In addition, TMT has been shown to produce effects on operant behavior since TMT-intoxicated rats had higher rates of lever pressing under a fixed-ratio schedule of food presentation[19], and TMT impaired the performance of differential reinforcement at low response rates in an operant schedule[19]. Moreover, TMT intoxication produces deficits in passive avoidance retention, but not in the acquisition of the passive avoidance response[14,20,22]. Furthermore, deficits in the acquisition of active avoidance at the beginning of training have been reported. These anatomical and behavioral findings have made TMT-intoxicated rats an attractive model for degenerative diseases such as AD, which is the most common cause of dementia[23].

The present study was designed to evaluate the protective effect of soybean-derived phosphatidylserine (SB-PS) on the TMT-induced learning and memory deficits in rats and to elucidate the mechanism underlying these protective effects. Rats were tested on a Morris water maze for spatial learning and memory. The analyzed parameters included the expression of cholinergic neurons and cAMP responsive element binding protein (CREB) and neural activity in the hippocampus.

Materials and Methods

1. Animals

Male Sprague-Dawley rats weighting 250-280 g each were purchased from Samtaco Animal Corp. (Kyungki-do, Korea). The rats were randomly divided into four groups each as follows: non-treated, nave normal group (Normal, n=11); TMT injection with vehicle(medium-chain triglyceride : MCT) administered group (MCT, n=6); TMT injection with 50 mg/kg SB-PS administered group (SB-PS 50, n=8); TMT injection with 100 mg/kg SB-PS administered group (SB-PS 100, n=4) used in this study. The animals were allowed to acclimatize themselves for at least 7 days prior to the experimentation. The animals were housed in individual cages under light-controlled conditions (12/12-hr light/dark cycle) and at 23℃ room temperature. Food and water were made available ad libitum. All the experiments were approved by the Kyung Hee University institutional animal care and use committee. Also, This experimental protocol was approved by an Institutional

Review Committee for the use of Human or Animal Subjects or that procedures are in compliance with at least the Declaration of Helsinki for human subjects, or the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC). The rats were allowed at least 1 week to adapt to their environment before the experiments.

2. Chemical treatment

SB-PS were supplied by Doosan Co. Glonet BU (Youngin, Korea). The applied SB-PS formula contains 90% phosphatidylserine (PS), 2% phosphatidylcholine (PC), and 6% phosphatidic acid (PA). The compositions of SB-PS are palmitic (17.7%), palmitoleic (1.3%), stearic (1.3%), oleic (14.4%), limoleic (61.2%), linolenic (1.4%), EPA and others (9.4%). The rats were injected intraperitoneally (i.p.) with TMT (8.0 mg/kg, body weight) dissolved in 0.9% saline and then they were returned to their home cages. The rats were orally administrated vehicle (MCT) or SB-PS (50, 100 mg/kg), daily for 21 days. From the 16th after the injection of TMT, the Morris water maze test was performed for 5days.

3. Morris water maze test

The swimming pool of the Morris water maze was a circular water tank 200 cm in diameter and 35 cm deep. It was filled to a depth of 21 cm with water at 23℃. A platform 15 cm in diameter and 20 cm in height was placed inside the tank with its top surface being 1.5 cm below the surface of the water. The pool was surrounded by many cues that were external to the maze[24]. A CCD camera was equipped with a personal computer for the behavioral analysis. Each rat received four daily trials. For 4 consecutive days, the rats were tested with three acquisition tests. They also received retention tests on the 5th day. For the acquisition test, the rat was allowed to search for the hidden platform for 180s and the latency to escape onto the platform was recorded. The animals were trained to find the platform that was in a fixed position during 4 days for the acquisition test, and then for the retention test (at the 5th day), they received a 1 min probe trial in which the platform was removed from the pool. The inter-trial interval time was 1 min. The performance of the test animals in each water maze trial was assessed by a personal computer for the behavioral analysis (S-mart program, Spain).

4. Immunohistochemistry

Briefly, the rats were anesthetized (sodium pentobarbital, 100 mg/kg, i.p.) then perfused transcardially with phosphate-buffered saline (PBS; pH 7.4) for 30s followed by 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 10-15 min. The brains were postfixed in the same fixative overnight, cryoprotected in 30% sucrose solution in PBS, embedded and serially sectioned on a cryostat (Leica, Germany) at 30 m thickness in the coronal plane and they were collected in PBS. The primary antibodies against the following specific antigen were used: Cholinacetyl transferase (sheep polyclonal ChAT, concentration 1:2000; Cambridge Research Biochemicals, Wilmington, D.E.), acetylcholine esterase (rabbit polyclonal AChE, concentration 1:200; Santacruz biotechnology, Delaware Avenue Santa Cruz, CA, U.S.A) and cAMP responsive element binding protein (rabbit polyclonal CREB, concentration 1:250; Cell Signaling, Boston, U.S.A.). The primary antibody was prepared and diluted in 0.2% PBST, 2% blocking serum and 0.001% kehole limpit hemocyanin (Sigma, U.S.A.). The sections were incubated in the primary antiserum for 72h at 4℃. After three more rinses in PBST, the sections were placed in Vectastain Elite ABC reagent (Vector laboratories, Burlingame, CA) for 2h at room temperature. Following a further rinsing in PBS, the tissue was developed using diaminobenzadine (Sigma, USA) as the chromogen. The images were captured using a DP2-BSW imaging system (Olympus, CA, USA) and they were processed using Adobe Photoshop. For measuring the cells that were positive for ChAT, AChE and CREB, the grid was placed on CA1 and CA3 in the hippocampus area according to the method of Paxinos G. et al[25]. The number of cells was counted at 100 x magnification using a microscope rectangle grid that measured 200 x 200um^2. The cells were counted in three sections per rat within the hippocampus.

5. F-18 FDG micro PET scan

All the rats were deprived of food for 12-15 h before the experiments to enhance the F-18 FDG uptake in the brain. Each animal was placed on a heating pad in a cage and warmed for at least 30 min before the F-18 FDG injection. The temperature of the cages was kept at 30℃ throughout the uptake period in accordance with an optimized F-18 FDG uptake protocol[26]. F-18 FDG (500 Ci/100 g body weight) was injected through a tail vein and the rats were anesthetized with 2% isoflurane in 100% oxygen (Forane solution; ChoongWae Pharma). For the PET imaging, a Siemens Inveon PET scanner (Siemens Medical Solutions, USA) was used throughout the study. The transverse resolution that was used was < 1.8 mm at the center[27,28].

The transmission PET data was acquired for 15 min

using a Co-57 point source with an energy window of 120-125 keV. One mCi of F-18 FDG was injected. After allowing for 30 min of tracer uptake time, 30 min of emission PET data was acquired within an energy window of 350-650 keV. The emission list-mode PET data was sorted into 3D sinograms and reconstructed using 3 DRP methods. The pixel size of the reconstructed image was 0.15 0.15 0.79 mm^3. Attenuation and scatter corrections were performed for all the datasets[29].

6. Voxel based statistical analysis

Voxel based statistical analysis was performed to compare the cerebral glucose metabolism of the SB-PS group and MCT group datasets. The procedure used for SPM analysis of the animal PET data was as previously described in our previous study[30].

Briefly, for efficient spatial normalization, only the brain region was extracted. A study specific template was then constructed using all the datasets. The PET data was spatially normalized onto a rat brain template and smoothed using a 3 mm Gaussian kernel. Count normalization was performed. A voxel-wised t-test between the SB-PS and MCT datasets was performed using the Statistical Parametric Mapping 5 program ($P < 0.005$, $K > 50$)

7. Statistical analysis

Statistical comparisons were done for the behavioral and histochemical studies using one-way and repeated measures of ANOVA, respectively and LSD test was done. All of the results are presented as means S.E.M., and I used SPSS 15.0 for Windows for analysis of the statistics. The significance level was set at $P<0.05$.

Results

1. Effect of SB-PS on the performance of the water maze test

The effect of SB-PS on spatial learning was evaluated on the Morris water maze test. As shown in Fig. 1a, the escape latency of the MCT group was longer by means of memory impairment than that of the normal group during all the trial session. The escape latency differed among the groups when the results were averaged over all the session. The MCT group showed a worse performance than did the normal group (at the Day[1,2,4]. In acquisition test, SB-PS 50 group produced a significant improvement of the escape latency to find the platform from the 2nd day ($p<0.01$)and 4th day ($p<0.05$) In addition, SB-PS 100 produced a significant improvement of the escape latency to find the platform from the all day ($p<0.01$). To examine the spatial memory of rats, the time spent swimming to the platform was compared and the analysis is illustrated in Fig. 1b. The times spent on the platform were significantly different among the groups ($F3,28=6.2$, $p<0.01$) the MCT group spent less time around the platform than the normal group. However, SB-PS groups showed increased time spent around the platform compared to that of the MCT group (SB-PS 50 $p<0.05$, SB-PS 100 $p<0.01$)(Fig. 1a and b)

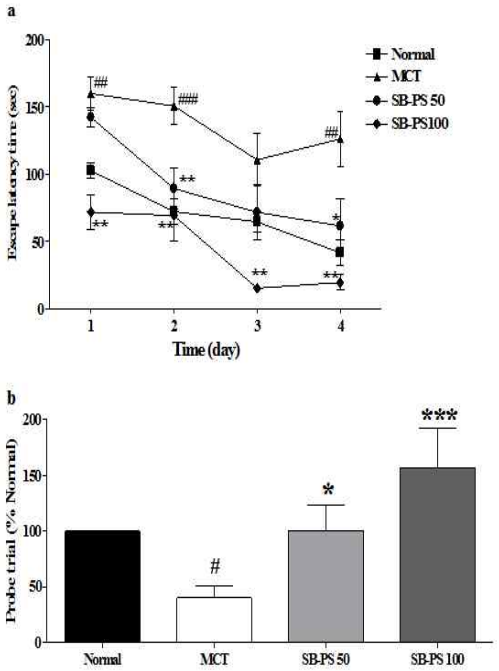

Fig. 1. a. The latency to escape onto the hidden platform during the Morris water maze. The task was performed with 3 trials per day during 4 days for the acquisition test. b. Retention performance was tested on 5th day. The rats received a 1 min probe trial in which the platform was removed from the pool for retention testing. (The values are presented as means S.E.M. #p<0.05, ##p<0.01, ###p<0.001 vs. normal group & *p<0.05, **p<0.001 vs. MCT group, respectively.)

2. Cholineacetyl transeferase (ChAT) immunoreactivity in the hippocampus

The results of the evaluations of the ChAT immunoreactive cells per section from the different hippocampal formations are shown in Fig. 2a and b Post-hoc comparisons indicated that the ChAT activity in the hippocampus of the MCT group was significantly lower than that of the normal group ($p<0.001$). In particular, there were significant differences in both CA1 ($F3,19=27.1$, $p<0.001$) and CA3 ($F3,21=11.9$, $p<0.001$). However, the ChAT reactivity in the SB-PS 50 group was higher than that of the MCT group, and particularly in CA1 ($p<0.001$) and CA3 ($p<0.01$). However, the ChAT reactivity in the hippocampus of the SB-PS 100 group showed no statistically significantly differences among the groups, but there was a slight trend for a significant interaction effect on the expression of ChAT in the

hippocampus(Fig. 2a and b).

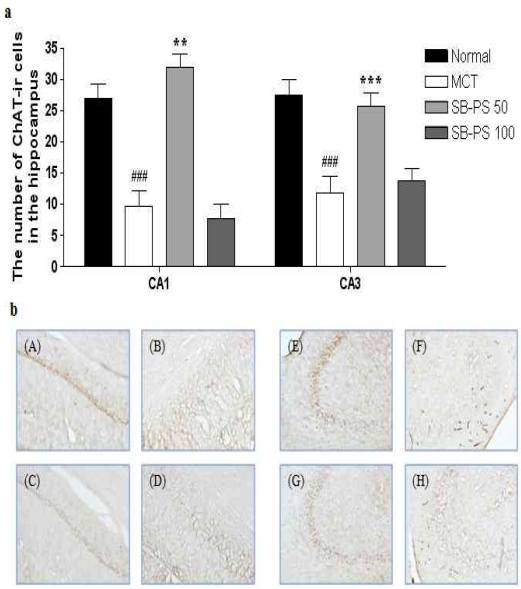

Fig. 2. a. The number of choline acetyltransferase (ChAT) immunostained nuclei in different hippocampal CA1 and CA3 of the experimental groups. Each values represents the S.E.M.(###p<0.001 compared to the normal and **p<0.01, ***p<0.001 compared to the MCT group.) b. Photographs showing the distribution of ChAT-immunoreactive cells in the hippocampus of normal group (A,E), MCT group (B,F), SB-PS 50 group (C,G) and SB-PS 100 group (D,H). Sections were cut coronally at 30 m and the scale bar represents 200 m.

3. Acetylcholinesterase (AChE) immunoreactivity in the hippocampus

The results of the evaluations of the AChE immunoreactive cells per section from the different hippocampal formations are shown in Fig. 3a and b Post-hoc comparisons indicated that the AChE activity in the hippocampus of the MCT group was significantly lower than that of the Normal group ($p<0.05$). In particular, there were significant differences in both CA1 ($F_{4,22}=7.3$, $p<0.001$) and CA3 ($F_{4,24}=7.6$, $p<0.05$). However, the AChE reactivity in the SB-PS 50 group was higher than that of the MCT group, and particularly in CA1 ($p<0.05$) and CA3 ($p<0.01$). However, the AChE reactivity in the hippocampus of the SB-PS 100 group showed no statistically significantly differences among the groups, but there was a slight trend for a significant interaction effect on the expression of AChE in the hippocampus(Fig. 3a and b).

4. Cyclic AMP responsive element binding protein (CREB) immunoreactivity in the hippocampus

The results of the evaluations of the CREB immunoreactive cells per section from the different hippocampal formations are shown in Fig. 4a and b Post-hoc comparisons indicated that the CREB activity in the hippocampus of the MCT group was significantly lower than that of the normal group ($p<0.05$). In particular, there were significant differences in the hippocampal CA1 ($F_{3,25}=6.6$, $p<0.05$) and CA3 ($F_{3,24}=6.6$, $p<0.01$). The CREB reactivity in the SB-PS treated group was higher than that of the MCT group and particularly in hippocampus, the number of CREB positive neurons in the SB-PS 50 group was significantly increased by MCT group, and particularly in CA1 ($p<0.001$) and CA3 ($p<0.01$). In addition SB-PS 100 group was significantly increased by MCT group, and particularly in CA3 ($p<0.01$)(Fig. 4a and b)

5. Change in brain glucose metabolism

The results of the evaluations of the FDG uptake from the different brain regional formations are shown in Fig. 5a, b and c. FDG-PET image scans indicated differences in the cerebral metabolic rate of glucose from the hippocampus to prefrontal cortices between the MCT group and SB-PS groups. On the SPM analysis, the cerebral glucose metabolism of the MCT group datasets was significantly decreased in the frontal lobe compared to the normal group ($p<0.05$). However, the cerebral metabolic rate of glucose in the SB-PS treated groups were markedly increased in frontal lobe ($p<0.001$) and hippocampus ($p<0.001$)(Fig. 5a, b and c).

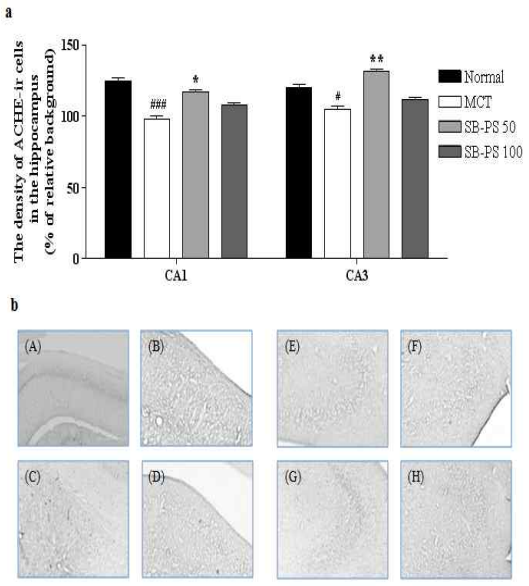

Fig. 3. a. The number of acetylcholine esterase (AChE) immunostained nuclei in different hippocampal CA1 and CA3 of the experimental groups. Each values represents the S.E.M. #(p<0.05, ##p<0.001 compared to the Normal and *p<0.05, **p<0.01 compared to the MCT group.) b Photographs showing the distribution of AChE-immunoreactive cells in the hippocampus of normal group (A,E), MCT group (B,F), SB-PS 50 group (C,G) and SB-PS 100 group (D,H). Sections were cut coronally at 30 m and the scale bar represents 200 m.

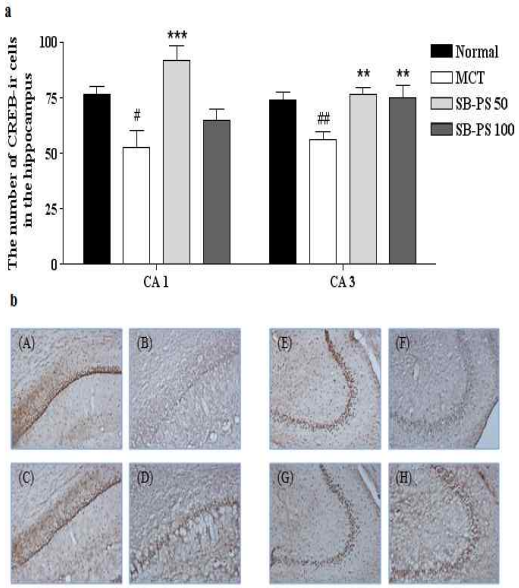

Fig. 4. a The number of cAMP responsive element binding protein (CREB) immunostained nuclei in different hippocampal CA1 and CA3 of the experimental groups. Each values represents the S.E.M. (#p<0.05, ##p<0.01 compared to the normal group and **p<0.01, ***p<0.001 compared to the MCT group.) b. Photographs showing the distribution of CREB-immunoreactive cells in the hippocampus of normal group (A,E), MCT group (B,F), SB-PS 50 group (C,G) and SB-PS 100 group (D,H). Sections were cut coronally at 30 m and the scale bar represents 200 m.

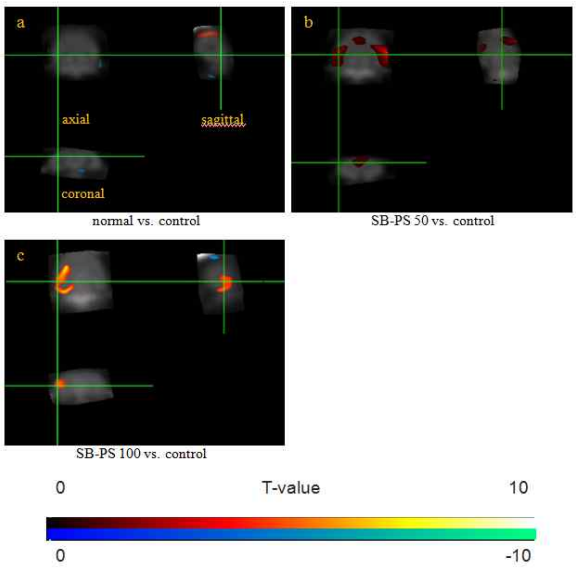

Fig. 5. Changed regional (Frontal lobe, Hippocampus) FDG uptake in the rat brain. The colored area represents a significant difference in glucose metabolism.

Discussion

The present study demonstrated that TMT injections produced severe deficits in the rats' performances in a Morris water maze along with signs of neuro-degeneration, including decreased cholinergic neurons and cAMP responsive element binding protein (CREB) activity in the hippocampus. Repeated treatment with SB-PS attenuated the TMT-induced learning and memory deficits in the water maze test and it had a protective effect against the TMT-induced decrease in cholinergic and CREB positive neurons. TMT intoxication impairs the acquisition of water maze performance[19]. The Morris water maze is a well-established paradigm for evaluating deficits in hippocampal-dependent memory and the MWM spatial learning task has been used in the validation of rodent models of neurocognitive disorders and for the evaluation of possible neurocognitive treatments[24,31]. The impairment in spatial learning produced by TMT in the current study is consistent with the previous reports of spatial learning impairments[19-21]. In this current study proved that spatial memory continued to improve in the SB-PS groups during the training days compared to that of the MCT group. Also, the data of the spatial probe trial demonstrated that SB-PS protects against the TMT-induced decrease of the spatial retention, and especially long-term memory. It has been previously reported that SB-PS has profound curative effects on improving the memory and cognitive function of an elderly people with mild cognitive impairment[32]. It has been previously reported that SB-PS has profound curative effects on improving the memory and cognitive function of an aged rats

The neuroprotective effects of these natural drugs on the central acetylcholine system were also examined by performing immunohistochemistry of the hippocampal neurons. The degeneration of the cholinergic innervation from the basal forebrain to the hippocampal formation in the temporal lobe is thought to be one of the factors determining the progression of memory decay, both during normal aging and AD[33]. The best available marker for cholinergic neurons in the basal forebrain is ChAT activity. ChAT synthesizes the neurotransmitter acetylcholine in the basal forebrain, cortex, hippocampus and amygdala. A significant reduction in ChAT activity in the postmortem brains of demented patients has been reported. In addition, there was a 50-60% decrease in ChAT activity in the hippocampus of the TMT-induced rats in this current study. However, the present results show that SB-PS had a significant interaction effects on cholinergic neurotransmission in the brain by increasing the hippocampus ChAT activities. Moreover, there was a 10-20% decrease in AChe-ir activity in the hippocampus of the TMT-induced rats in this current study. However, the present results show that SB-PS exerts beneficial effects effects on cholinergic neurotransmission in the brain by increasing the hippocampus AChe-ir activities.

Many studies have indicated that disruption or deficiency

of the CREB gene leads to neurodegeneration[34]. CREB is also a molecular marker of long term potentiation and memory formation. Previous studies have proved that the CREB mutation affected learning and memory, and the mutant gene disrupted long term memory and hippocampus-dependent tasks[35]. CREB is critical for activating the transcription of genes controlled by the cAMP-response element, and many of these genes may be involved in neuronal growth and plasticity and they may take part in neuronal survival[36]. Genetic and pharmacological studies have provided strong evidence that the CREB signaling pathway is crucial for learning and memory across species[37]. Consistent with the previous studies, our results showed that the levels of CREB in the hippocampus showed significant differences among the groups. The TMT treated group showed a reduction, by approximately 20~30%, of the CREB activity in the hippocampus in this current study. Thus, I may draw a conclusion that the CREB loss after TMT exposure might be at least partially responsible for the TMT-induced cell death. It has been reported that CREB could be inactivated by stressful stimuli such as zinc deficiency or hypoxia[34,38]. Our results indicated that TMT played a role as stressful stimulation on the CREB gene. However, the CREB expression was significantly up-regulated after SB-PS treatment in this experiment. Perhaps the activation of CREB was related to a neuroprotective effect such as a defense mechanism.

On the PET analysis, the cerebral glucose metabolism of the SB-PS datasets was significantly increased in the hippocampus and frontal lobe as compared to that of the MCT. An obvious limitation of our study is that the spatial resolution of the present micro PET system is not high enough to permit more specific analysis of the activity changes within certain brain structures. Nevertheless, there have been several studies that have investigated the brain activity changes in small animals using micro PET technology[39]. Thus, an important point of our study is that in spite of the limited spatial resolution of the micro PET system.

In summary, treatment with SB-PS attenuated the TMT-induced learning and memory deficits in the Morris water maze, and SB-PS treatment had a protective effect against a TMT-induced decrease of the cholinergic neurons and CREB activation. Treatment with SB-PS also significantly improved the glucose activity of the hippocampus. Thus, SB-PS is a good candidate for further investigations that may ultimately result in its clinical use. Further studies that will examine the effects of SB-PS activation on additional behavioral tasks will help to elucidate whether increasing the CREB signaling may also improve other types of memory.

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