Sesaminol Glucosides Protect β-Amyloid Peptide-Induced Cognitive Deficits in Mice

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This study was designed to investigate the effect of sesaminol glycosides (SG), one of the most abundant lignan glycosides in sesame (Sesamum indicum Linn.) seed, on cognitive deficits and oxidative stress induced by intracerebroventricular (i.c.v.) injection of β-amyloid protein (Aβ25–35) in mice. Mice were fed diets containing 0%, 0.25%, or 0.5% of SG for six weeks. Dietary SG showed a protective effect against Aβ-induced learning and memory deficits in passive avoidance and the Morris water maze test. Aβ caused significant neuronal loss in the CA1 and CA3 regions of the hippocampus, but SG supplement showed decrease of the Aβ25–35-induced neuronal loss. The SG supplementation significantly decreased thiobarbituric acid reactive substance values and 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels in brain tissue. SG also reversed the activity of glutathione peroxidase (GPx), which is decreased by Aβ. These results suggest that SG protects against cognitive deficits induced by Aβ25–35, in part through its antioxidant activity.

Key words: sesame; sesaminol glycoside; β-amyloid peptide25–35; cognitive deficit; oxidative stress

Alzheimer’s disease (AD) is characterized by progressive cognitive function deficits due to the presence of numerous senile plaques and neurofibrillary tangles in certain brain regions.1) The main component of senile plaques is β-amyloid peptide (Aβ), which is derived from a large amyloid precursor protein (APP).2) Aβ25–35 contains the residues important for aggregation and toxicity including methionine 35.3) This amyloid fragment has been shown to induce neurotoxicity in vitro as well as in vivo.4,5) In addition, the intracerebroventricular (i.c.v.) administration of Aβ25–35 into rodent brain induced histological change and cognitive deficit.6,7) The deposition of Aβ may cause neuronal death via a number of possible mechanisms including oxidative stress and inflammatory response, which has been suggested to play a key role in AD pathogenesis.8

Oxidative stress has been hypothesized to play a pivotal role in excitotoxicity, inflammation, and apoptosis in the AD brain. Marker of oxidative stress such as lipid peroxides, protein carbonyl groups, and 8-hydroxy-2′-deoxyguanosine (8-OHdG) are increased in the brains of AD patients than in aged-matched control brains.8,9) Moreover, it has been reported that Aβ induces neurotoxicity via oxidative stress, and some antioxidants, such as vitamin E and ferulic acid, protect neurons from Aβ-induced apoptosis.4,10,11) Therefore, the attenuation of oxidative stress through antioxidant therapies may be useful in the treatment/prevention of AD.12

Sesame (Sesamum indicum Linn.) is widely known as a natural healthy food, and contains sesame lignin and lignan glycosides.13) Sesame lignan compounds, including sesamin glucosides (SG), have been shown to inhibit endogenous lipid peroxidation as well as oxidative DNA damage in rat liver and kidney.14) These compounds also scavenge peroxyl-radicals in in vitro experimental systems.15–17) Kang et al.18) have reported that defatted sesame flour containing SG inhibits lipid peroxidation in hypercholesterolemic rabbits. We also observed that SG had a protective effect against Aβ induced neuronal cell death19) and showed an inhibitory effect on lipopolysaccharide (LPS)-induced inflammatory response in astrocytes.20) Taking those facts into account, we hypothesized that SG exerts a neuroprotective effect in AD.

Since the effect of SG on cognitive function has not been reported, we investigated whether SG can exhibit the protective effect on the Aβ25–35-induced cognitive impairments.

MATERIALS AND METHODS

Sample Preparation SG isolated form sesame seed was prepared according to the method described by Katsuaki et al.13) Defatted sesame flour was extracted with distilled water at 95°C for 1 h to obtain a crude extract containing sesaminol glucosides. Crude SG was further purified by Diaion HP 20 (Mitsubishi Chemicals, Co., Tokyo, Japan) column using 60% ethanol. The extracts were concentrated under reduced pressure and freeze-dried for storage until use. Total SG content of the extract was determined by UV spectrosopy, and the composition was determined using HPLC. SG have a purity of approximately 70 to 80%.

Animals and Diets All animal procedures were approved by Institutional Animal Care and Use Committee of Korea Food Research Institute. Forty male ICR mice (5 weeks old; Charles River Laboratories Inc.; Jung-Ang Lab. Animal, Inc., Korea) were randomly divided into four groups (n = 10). Mice were allowed free access to water, but were provided with different diets for six weeks. The experimental diet was based on the AIN-76 formula, and comprised either 0.25% or 0.5% SG. The mice were housed in a room maintained at 23 ± 1°C with a 12-h light/dark cycle.

Intracerebroventricular (i.c.v.) Injection of Aβ25–35 Aβ25–35 (Sigma-Aldrich Co., CA, U.S.A.) was dissolved in phosphate-buffered saline (PBS) and injected into animals at the beginning of each behavioral test trial by i.c.v. (10 µg/10 µl/mouse) according to the procedure established by Lauren and Belknap.21) Briefly, each mouse was injected at the bregma with a 50 µl Hamilton microsyringe fitted with...
a 26-gauge needle, the tip of which was adjusted for an insertion depth of 2.4 mm. Animals injected with PBS (vehicle) alone served as a control. The schematic of experimental schedule was shown in Fig. 1.

**Passive Avoidance Test** The day before Aβ25-35 injection (at beginning of 5 weeks), mice were trained on a passive avoidance task. Passive avoidance was tested using a two-compartment shuttle chamber (256000 series, TSE Systems, Germany), one illuminated and one dark, equipped with a grid floor and shock generator. During the training trial, each mouse was placed in the lit compartment, and as soon as it entered the dark compartment, the door was closed and it received an inescapable shock (0.3 mA, 3 s). The day after Aβ25-35 injection, the mouse was again placed in the lit compartment, and the time until it returned to the dark compartment was measured as the step-through latency (with a maximum of 300 s). If the mouse did not enter the dark compartment within 300 s, the mouse was assumed to have memorized the passive avoidance training after one training trial.

**Morris Water-Maze** The Morris water-maze was performed as described previously. The experimental apparatus consisted of circular water tank (diameter, 100 cm; height, 35 cm) containing water at 23 °C at a depth of 15 cm and rendered opaque by the addition of powdered milk. A transparent platform was positioned inside the tank such that its top was submerged 2 cm below the water surface. The first training day dedicated to swimming training for 60 s in the absence of the platform. During the four subsequent days the mice were given three trial sessions per day with the platform in place. When a mouse located the platform, it was permitted to remain on it for 10 s. If the mouse did not locate the platform within 120 s, it was placed on the platform for 10 s. In each training trial, the time required to find the hidden platform was recorded. After several trials, the probe trial was conducted after the second injection of Aβ25-35 (at beginning of 6 weeks).

**Histology** After behavioral test, the mice were anesthetized with ethyl ether and perfused transcardially with PBS followed by 4% paraformaldehyde for fixation. The brains were removed and further fixed in 4% paraformaldehyde overnight at 4 °C. The fixed brain was cut into 30 μm sections on a sliding microtome, and the section stained with hematoxylin and eosin (HE).

**Measurement of DNA Damage in the Brain** The degree of DNA damage in the brain tissue was estimated from the concentration of 8-hydroxy-2’-deoxyguanosine (8-OHdG). Brain tissue DNA fractions were obtained using a DNA Extractor WB kit (Wako Pure Chemical Industries Ltd., Tokyo, Japan). Nuclear DNA was hydrolyzed with nuclelease P1 (Yamasa Corporation, Choshi, Japan) and alkaline phosphatase (Roche, Mannheim, Germany) and quantified using a competitive enzyme-linked immunosorbent assay (ELISA) kit for detection of 8-OHdG (New 8-OHdG Check, Japan Institute for the Control of Aging, Japan).

**Lipid Peroxidation** Lipid peroxidation in the brain tissue was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) as described by Ohkawa et al.

**Antioxidant Enzyme Activity** The brain tissue was homogenized in 10 volumes (w/v) of buffer containing 0.25 M ice-cold sucrose and 0.5 mM ethylenediamine tetraacetic acid (EDTA), and the homogenate was centrifuged at 600 g (VS-500N, VISION Scientific, Ltd., Korea) for 10 min at 4 °C. An aliquot of the supernatant was collected for the assay of catalase activity. The supernatant was then centrifuged at 10000×g (RC 5C plus, Sorvall, U.S.A.) for 30 min at 4 °C. The resulting supernatant was used for assay of superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity. The activities of catalase, SOD, and GPx in the brain were measured according to Aebi,24) Marklund and Marklund,25) and Lawrence and Burk,26) respectively. The protein content of the supernatant was determined by the method of Lowry et al. with serum bovine albumin as the standard.

**Statistics** All results are expressed as means±S.E. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by post-hoc Tukey analysis. The statistical significance was set at p<0.05.

**RESULTS**

**Effect of SG on Aβ25-35-Induced Cognitive Deficits in Mice** In our preliminary study, administration of Aβ25-35 and Aβ1-40 reduced step-through latency in passive avoidance test. But, no significant difference between the two groups was observed (data not shown). Considering this fact, we performed experiments with Aβ25-35.

Changes in the step-through latency in passive avoidance are shown in Fig. 2A. In the retention trial, i.e. v. administration of Aβ25-35 significantly reduced step-through latency. However, dietary supplements of SG significantly ameliorated the Aβ25-35-induced cognitive deficits, and this im-

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Fig. 1. Schematic of the Experimental Schedule

Animals were maintained on a SG-free diet or a SG-supplemented diet for six weeks. Two groups of mice were provided with a diet containing either 0.25% or 0.5% SG for six weeks. Other groups were given the same feed but without SG. Animals received injections of Aβ25-35 at the indicated times. Aβ25-35 was administrated to three of the groups (those receiving SG and one of the non-SG-fed groups) at the beginning of 5 weeks and 6 weeks. The remaining non-SG-fed group was administrated vehicle alone at the same times (control group).
provement was seen in a dose-dependent manner.

In Morris water maze tests, control animals learned the location of the hidden platform more rapidly than Aβ25–35-receiving animals, with a more than two-fold difference in escape latency of 5th day between these groups (Fig. 2B). However, administration of a 0.25% or 0.5% SG diet for six weeks restored the escape latency of 5th day to near normal levels. No significant differences were found in swim speed or in the escape latency among the four groups during the course of four consecutive training trials.

**Effect of SG on Morphologic Alterations Induced by Aβ25–35**

The effect of SG on the morphologic alterations of hippocampus regions was observed using HE staining method (Fig. 3). No remarkable neuronal abnormalities were observed in brains from mice injected with vehicle, but the Aβ25–35 administration caused selective neuronal loss in the hippocampal CA1 and CA3 region. In contrast, SG markedly reduced the damaged pyramidal cells.

**Effect of SG on Aβ25–35-Induced Oxidative DNA Damage and Lipid Peroxidation in the Brain**

The effect of SG on Aβ25–35-induced oxidative stress was assessed by measuring 8-OHdG levels and lipid peroxidation in brain tissue. As shown in Fig. 4, Aβ25–35 increased 8-OHdG levels by two-fold. This increase in oxidative DNA damage was completely blocked in mice maintained on a 0.5% SG diet.

Aβ25–35 increased not only oxidative DNA damage but also lipid peroxidation, as measured by thiobarbituric acid reactive substance (TBARS) levels (Fig. 5). This increase was not significantly affected by a 0.25% SG diet, but was completely attenuated by a 0.5% SG diet.

**Effect of SG on Antioxidant Defenses Following Aβ25–35**
together, these results suggest that SG protects neurons from exposure to Aβ. 

Inhibits neuronal apoptosis induced by Aβ. We have previously reported that SG also inhibits neuronal apoptosis induced by Aβ. Studies showing that Aβ suppresses ROS and nitric oxide generation, and antioxidant compounds rescue neuronal cells from Aβ toxicity. We have previously shown that SG ameliorates Aβ-induced cytotoxicity and alters expression of apoptosis-related proteins such as bax, caspase, and poly(ADP-ribose) polymerase (PARP) in PC12 cells. We have also shown that SG suppresses ROS and nitric oxide generation, NF-κB activation, and pro-inflammatory gene expression induced by lipopolysaccharide (LPS) in cultured rat astrocytes. SG has also been shown to have a host of other physiological effects including anti-inflammatory and anti-carcinogenic effects. Our results, taken with these findings, suggest that the anti-apoptotic properties of SG may be related to its anti-inflammatory effects. Aβ has been shown to induce apoptotic neuronal death through generation of oxidative stress, and antioxidant compounds rescue neuronal cells from Aβ toxicity. We have previously shown that SG ameliorates Aβ-induced cytotoxicity and alters expression of apoptosis-related proteins such as bax, caspase, and poly(ADP-ribose) polymerase (PARP) in PC12 cells. We have also shown that SG suppresses ROS and nitric oxide generation, NF-κB activation, and pro-inflammatory gene expression induced by lipopolysaccharide (LPS) in cultured rat astrocytes. SG has also been shown to have a host of other physiological effects including anti-inflammatory and anti-carcinogenic effects. Our results, taken with these findings, suggest that the anti-apoptotic properties of SG account for the ability of SG prevent Aβ-induced learning and memory deficits.

In summary, SG protects against cognitive deficits induced by Aβ in mice, and this effect may be mediated by its antioxidant properties. However, in this study, whole brain, nor cortex and hippocampus that mainly related learning and memory functions, was used because their amount to measure various biomarkers was insufficient. Hence, identification on various brain regions was performed to further understand. SG may hold promise in the treatment of AD, but the precise mechanism underlying the cognitive performance.
enhancing activity of SG should be studied further.

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REFERENCES