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s-Allyl Cysteine, *s*-Ethyl Cysteine, and *s*-Propyl Cysteine Alleviate β-Amyloid, Glycative, and Oxidative Injury in Brain of Mice Treated by D-Galactose

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ABSTRACT: The neuroprotective effects of *s*-allyl cysteine, *s*-ethyl cysteine, and *s*-propyl cysteine in D-galactose (DG)-treated mice were examined. DG treatment increased the formation of Aβ_{1-40} and Aβ_{1-42}, enhanced mRNA expression of β-amyloid precursor protein (APP) and β-site APP cleavage enzyme 1 (BACE1), and reduced neprilysin expression in brain ($P < 0.05$); however, the intake of three test compounds significantly decreased the production of Aβ_{1-40} and Aβ_{1-42} and suppressed the expression of APP and BACE1 ($P < 0.05$). DG treatments declined brain protein kinase C (PKC) activity and mRNA expression ($P < 0.05$). Intake of test compounds significantly retained PKC activity, and the expression of PKC-α and PKC-γ ($P < 0.05$). DG treatments elevated brain activity and mRNA expression of aldose reductase (AR) and sorbitol dehydrogenase as well as increased brain levels of carboxymethyllysine (CML), pentosidine, sorbitol, and fructose ($P < 0.05$). Test compounds significantly lowered AR activity, AR expression, and CML and pentosidine levels ($P < 0.05$). DG treatments also significantly increased the formation of reactive oxygen species (ROS) and protein carbonyl and decreased the activity of glutathione peroxidase (GPX), superoxide dismutase (SOD), and catalase ($P < 0.05$); however, the intake of test compounds in DG-treated mice significantly decreased ROS and protein carbonyl levels and restored brain GPX, SOD, and catalase activities ($P < 0.05$). These findings support that these compounds via their anti-Aβ, antiglycative, and antioxidative effects were potent agents against the progression of neurodegenerative disorders such as Alzheimer's disease.

KEYWORDS: β-Amyloid, *s*-allyl cysteine, *s*-ethyl cysteine, *s*-propyl cysteine, glycation, protein kinase C

INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the most common form of dementia in many countries. The "amyloid cascade hypothesis" considers the amyloid-β peptide (Aβ) a central role in the pathogenesis of AD.[1,2] Aβ is derived from the β-amyloid precursor protein (APP), a large transmembrane protein, in which a transmembrane aspartyl protease termed as the β-site APP cleavage enzyme 1 (BACE1) is responsible for the cleavage.[3,4] On the other hand, Aβ could be degraded by a variety of proteases such as neprilysin (NEP).[5] Thus, any agents with the ability to down-regulate APP or BACE1, or up-regulate NEP, may potentially protect neurons against Aβ-induced neurotoxicity via decreasing Aβ formation and/or increasing Aβ clearance. In addition, it has been documented that Aβ deposition in brain tissue causes the generation of free radicals and advanced glycation end products (AGEs) such as carboxymethyllysine (CML) and pentosidine, which in turn enhances oxidative and glycative damage and consequently leads to neuronal cells apoptosis.[6,7] Thus, any agents with antiglycative and antioxidative effects may also attenuate AD development.

s-Allyl cysteine (SAC), *s*-ethyl cysteine (SEC), and *s*-propyl cysteine (SPC) are hydrophilic cysteine-containing compounds naturally formed in *Allium* plants such as garlic and onion.[8] Our previous study has reported that preintake of SEC and SPC markedly protected mice brain against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson-like oxidative and inflammatory injury via increasing retention of dopamine and glutathione (GSH), elevating glutathione peroxidase (GPX) mRNA expression, and diminishing tumor necrosis factor-α mRNA expression.[9] Our other study further observed that SEC and SPC protected PC12 cells against Aβ-caused oxidative and apoptotic damage via retainability of mitochondrial membrane potential and a decrease in DNA fragmentation.[10] These findings support that these cysteine-containing compounds, via acting as antioxidative and anti-inflammatory agents, are able to prevent or mitigate cytotoxicity and oxidative stress that occurs in neurodegenerative disorders. Therefore, an animal study was conducted to further examine the in vivo protective effects and possible action modes of these agents against AD progression.

It has been documented that D-galactose (DG) induced aging-related and/or AD-like pathological changes including the increase of reactive oxygen species (ROS) and the decrease of

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antioxidant enzyme activity in brain.[11,12] The animal study of Hsieh et al.[12] reported that DG treatment increased AGE levels in circulation and enhanced Aβ expression in brain. In our present study, DG-injected mouse was used as an AD model to examine the anti-Aβ, antiglycative, and antioxidative effects of SAC, SEC, and SPC.

MATERIALS AND METHODS

Chemicals. SEC (99.5%) was purchased from Aldrich Chemical Co. (Milwaukee, WI). SAC (99.5%) and SPC (99%) were supplied by Wakunaga Pharmaceutical Co. (Hiroshima, Japan).

Animals. Three to four week old male C57BL/6 mice were obtained from National Laboratory Animal Center (National Science Council, Taipei City, Taiwan). Mice were housed on a 12 h light−12 h dark schedule and fed with water and mouse standard diet for 16 weeks. Use of the mice was reviewed and approved by both Chung Shan Medical University and China Medical University animal care committees.

Experimental Design. Mice at 19−20 weeks old were used for experiments. Mice were divided into two groups, in which one group was treated with DG (100 mg/kg body weight) via ip daily injection, and the other group was treated with saline injection. Both DG- and non-DG-treated mice were further divided into four subgroups, in which water, SAC, SEC, or SPC was supplied. SAC, SEC, or SPC, each compound at 1 g/L, was directly added into the drinking water. The consumed water volume and body weight were recorded weekly. After 7 weeks of DG treatment and cysteine-containing compounds supplementation, mice were sacrificed by decapitation. The brain was quickly removed and collected. Brain tissue at 0.1 g was homogenized on ice in 2 mL of phosphate buffer saline (PBS, pH 7.2), and the filtrate was collected. The protein concentration of filtrate was determined by a commercial assay kit (Pierce Biotechnology Inc., Rockford, IL) with bovine serum albumin as the standard. In all experiments, the sample was diluted to a final concentration of 1 mg protein/mL.

Measurement of Aβ Levels. $A\beta_{1-40}$ and $A\beta_{1-42}$ were measured using commercial colorimetric ELISA kits (The Genetics Co., Schlieren, Switzerland) according to manufacturer's instructions. All values were standardized to the protein concentration of the sample.

Determination of BACE1 and NEP Activities. For BACE1 activity assay, brain tissue was homogenized and lysed in 100 μL of buffer (20 mM MES, pH 6.0, 150 mM NaCl, 2 mM EDTA, 5 μg/mL leupeptin, 0.2 mM PMSF, 1 μg/mL pepstatin A, 2 μg/mL aprotinin, and 0.5% Triton X-100). After it was centrifuged at 16000*g* for 20 min at 4 °C, 50 μL of lysate was incubated with reaction buffer containing 10 μM fluorogenic substrate, MOCAc-Ser-Glu-Val-Asn-Leu-Asp-Ala-Glu-Phe-Arg-Lys-Arg-Arg-NH2 (Peptide Institute, Inc., Osaka, Japan), at 37 °C for 1 h. The absorbance at 405 nm was measured on a fluorescence plate reader. The NEP activity was determined using the NEP-specific fluorogenic peptide substrate, *N*-dansyl-D-Ala-Gly-p-(nitro)-Phe-Gly (DAGPNG; Sigma Chem. Co., St. Louis, MO). Briefly, 50 μg of brain membrane protein was incubated with 50 μM DAGPNG and 20 μM elanapril dissolved in 0.5 mL of Tris-HCl, pH 7.4. Then, 0.8 mU leucine aminopeptidase was added to the reaction mixture and further incubated for 20 min at 37 °C. Following centrifugation at 3000*g* for 5 min, the supernatant was removed, and fluorescence (340 nm excitation, 520 nm emission) was read on a fluorescence plate reader.

Protein Kinase C (PKC) Activity Assay. Sample (100 μL) was incubated with a salt solution for 15 min in the presence or absence of 100 μM PKC-specific substrate and followed by adding 5 mg/mL digitonin and 1 mM ATP mixed with γ-[^{32}P]ATP (<1500 cpm/pmol). The reaction was stopped by 5% trichloroacetic acid, then spotted onto P81 phosphocellulose paper, and washed with phosphoric acid and acetone. The amount of incorporated radioactivity into the substrate was determined by scintillation counting. The PKC activity was normalized by the corresponding protein content.

Measurement of CML, Pentosidine, Sorbitol, and Fructose Contents. CML was immunochemically determined with an ELISA technique using the CML-specific monoclonal antibody 4G9 and calibration with 6-(*N*-carboxymethylamino)caproic acid (Roche Diagnostics, Penzberg, Germany). Intra- and interassay variability were 5.3 and 6.2%, respectively. Pentosidine was analyzed by a HPLC equipped with a C18 reverse-phase column and a fluorescence detector according to the method described in Miyata et al.[13] Briefly, sample was lyophilized and acid hydrolyzed in 500 μL of 6 N HCl for 16 h at 110 °C in screw-cap tubes purged with nitrogen. After neutralization with NaOH and dilution with PBS, the sample was used for HPLC measurement. In addition, 100 mg of brain was homogenized with PBS (pH 7.4) containing U-[^{13}C]-sorbitol as an internal standard. After the protein was precipitated by ethanol, the supernatant was lyophilized. The content of sorbitol and fructose in each lyophilized sample was determined by liquid chromatography with tandem mass spectrometry, according to the method of Guerrant and Moss.[14]

Activity of Aldose Reductase (AR) and Sorbitol Dehydrogenase (SDH). The method of Nishinaka and Yabe-Nishimura[15] was used to measure AR activity in brain by monitoring the decrease in absorbance at 340 nm due to NADPH oxidation. The SDH activity was assayed according to the method of Ulrich[16] by mixing 100 μL of homogenate, 200 μL of NADH (12 mM), and 1.6 mL of triethanolamine buffer (0.2 M, pH 7.4) and monitoring the absorbance change at 365 nm.

Determination of Malonyldialdehyde (MDA), ROS, Protein Carbonyl, and GSH Levels. MDA, an index of lipid peroxidation, was measured by using a commercial assay kit (OxisResearch, Portland, OR). The method described in Gupta et al.[17] was used to measure ROS level. Briefly, 10 mg of tissue was homogenized in 1 mL of ice cold 40 mM Tris−HCl buffer (pH 7.4) and further diluted to 0.25% with the same buffer. Then, samples were divided into two equal fractions. In one fraction, 40 μL of 1.25 mM 2′,7′-dichlorofluorescin diacetate in methanol was added for ROS estimation. Another fraction, in which 40 μL of methanol was added, served as a control for autofluorescence, which was determined at 488 nm excitation and 525 nm emission using a fluorescence plate reader. Protein carbonyls were determined with the Zentech PC kit (BioCell, Auckland, New Zealand). Briefly, 50 μL of sample was mixed with a 200 μL of dinitrophenylhydrazine (DNP) solution. The adsorbed DNP−protein was reacted with an anti-DNP−biotin antibody, followed by reacting with streptavidin-linked horseradish peroxidase probe and chromatin reagent. The absorbance at 450 nm was measured. The GSH concentration was determined by a commercial colorimetric GSH assay kit according to the manufacturer's instruction (OxisResearch). Reduced GSH was determined in this study.

Catalase, Superoxide Dismutase (SOD), and GPX Activity Assay. The activities of catalase, SOD, and GPX were determined by catalase, SOD, and GPX assay kits (Calbiochem, EMD Biosciences, Inc., San Diego, CA). The enzyme activity was expressed in U/mg protein.

Real-Time Polymerase Chain Reaction (RT-PCR) for mRNA Expression. Brain tissue was homogenized in guanidinethiocyanate, and total RNA was isolated using Trizol reagent (Invitrogen, Life Technologies, Carlsbad, CA). One microgram of RNA was used to generate cDNA, which was amplified using Taq DNA polymerase. PCR was carried out in 50 μL of reaction mixture containing Taq DNA polymerase buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 200 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 mM of each primer) and 2.5 U Taq DNA polymerase. The specific oligonucleotide primers of targets are shown in Table 1. The cDNA was amplified under the following reaction conditions: 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min. Twenty-eight cycles were performed for glyceraldehyde-3-phosphate

Table 1. Forward and Reverse Primers for Real-Time PCR Analysis

target	forward	reverse
APP	5′-GAC TGA CCA CTC GAC CAG GTT CTG-3′	5′-CTT GAA GTT GGA TTC TCA TAC CG-3′
BACE1	5′-TTG CCC AAG AAA GTA TTT GA-3′	5′-TGA TGC GGA AGG ACT GAT T-3′
NEP	5′-GAC CTA CCG GCC AGA GTA-3′	5′-AAA CCC GAC ATT TCC TTT-3′
PKC-α	5′-GAA CCA TGG CTG ACG TTT AC-3′	5′-GCA AGA TTG GGT GCA CAA AC-3′
PKC-β	5′-TTC AAG CAG CCC ACC TTC TG-3′	5′-AAG GTG GCT GAA TCT CCT TG-3′
PKC-γ	5′-GAC CCC TGT TTT GCA GAA AG-3′	5′-GTA AAG CCC TGG AAA TCA GC-3′
AR	5′-CCC AGG TGT ACC AGA ATG AGA-3′	5′-TGG CTG CAA TTG CTT TGA TCC-3′
SDH	5′-TGG GAG CTG CTC AAG TTG TG-3′	5′-GGT CTC TTT GCC AAC CTG GAT-3′
GAPDH	5′-TGA TGA CAT CAA GAA GGT GGT GAA G-3′	5′-CCT TGG AGG CCA TGT AGG CCA T-3′

Table 2. Water Intake, Body Weight, and Brain Weight of Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC. [a]

	water intake (mL/day/mouse)	body weight (g)	brain weight (g)
control	2.9 ± 0.4 a	29.5 ± 2.0 a	0.56 ± 0.08 a
SAC	2.5 ± 0.6 a	30.3 ± 1.4 a	0.58 ± 0.11 a
SEC	3.0 ± 0.5 a	29.0 ± 1.8 a	0.52 ± 0.06 a
SPC	2.7 ± 0.6 a	28.9 ± 2.2 a	0.60 ± 0.09 a
DG	3.1 ± 0.3 a	28.8 ± 1.7 a	0.53 ± 0.05 a
DG + SAC	2.4 ± 0.7 a	29.4 ± 1.9 a	0.57 ± 0.09 a
DG + SEC	2.8 ± 0.5 a	30.1 ± 1.3 a	0.59 ± 0.10 a
DG + SPC	2.9 ± 0.4 a	29.0 ± 2.1 a	0.56 ± 0.07 a

[a] Data are means ± SDs (n = 10). Means in a column without a common letter differ; $P < 0.05$.

dehydrogenase (GAPDH, the housekeeping gene), and 35 cycles were performed for others. Generated fluorescence from each cycle was quantitatively analyzed by using the Taqman system based on real-time sequence detection system (ABI Prism 7700, Perkin-Elmer Inc., Foster City, CA). In this study, the mRNA level was calculated as percentage of the control group.

Statistical Analysis. The effect of each treatment was analyzed from 10 different preparations (n = 10). Data were reported as means ± standard deviations (SDs) and subjected to analysis of variance (ANOVA). Differences among means were determined by the least significance difference test with significance defined at $P < 0.05$.

RESULTS

As shown in Table 2, DG treatment and intake of SAC, SEC, and SPC did not significantly affect water intake, body weight, and brain weight ($P > 0.05$). DG treatment significantly increased the formation of $A\beta_{1-40}$ and $A\beta_{1-42}$ in brain (Table 3, $P < 0.05$); however, the intake of three test compounds significantly decreased the brain level of $A\beta_{1-40}$ and $A\beta_{1-42}$ ($P < 0.05$). DG treatment enhanced mRNA expression of APP and BACE1 and reduced NEP expression in brain (Figure 1, $P < 0.05$). The intake of test compounds lowered APP and BACE1 expression ($P < 0.05$) but failed to affect NEP expression ($P > 0.05$). As shown in Table 4, DG enhanced BACE1 activity and decreased NEP activity ($P < 0.05$); the intake of test compound significantly reduced BACE1 activity only ($P < 0.05$). DG treatments also declined brain PKC activity and mRNA expression of PKC-α, PKC-β, and PKC-γ (Figure 2, $P < 0.05$). The intake of test compounds significantly retained PKC activity and the expression of PKC-α and PKC-γ ($P < 0.05$).

Table 3. Level (pg/mg Protein) of $A\beta_{1-40}$ and $A\beta_{1-42}$ in Brain from Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC[a]

	$A\beta_{1-40}$	$A\beta_{1-42}$
control	0.14 ± 0.03 a	0.10 ± 0.04 a
SAC	0.11 ± 0.04 a	0.12 ± 0.05 a
SEC	0.13 ± 0.05 a	0.11 ± 0.03 a
SPC	0.10 ± 0.02 a	0.09 ± 0.04 a
DG	2.43 ± 0.19 d	2.51 ± 0.21 c
DG + SAC	1.65 ± 0.13 c	1.42 ± 0.17 b
DG + SEC	1.57 ± 0.10 c	1.45 ± 0.10 b
DG + SPC	1.22 ± 0.11 b	1.32 ± 0.08 b

[a] Data are means ± SDs (n = 10). Means in a column without a common letter differ; $P < 0.05$.

The brain level of CML, pentosidine, sorbitol, and fructose was significantly increased in DG-treated mice (Table 5, $P < 0.05$). Three test compounds significantly reduced the levels of these parameters ($P < 0.05$). As shown in Table 6 and Figure 3, DG treatments elevated brain activity and mRNA expression of AR and SDH ($P < 0.05$); however, three test compounds significantly lowered AR activity and expression ($P < 0.05$) but did not affect SDH activity and expression ($P > 0.05$). As shown in Table 7, intake of test compounds significantly raised GSH content in brain from mice without DG treatment ($P < 0.05$). DG treatments led to a significant increase in the formation of MDA, ROS, and protein carbonyl and a decrease in GSH level ($P < 0.05$); however, the intake of test compounds in DG-treated mice significantly reduced MDA, ROS, and protein carbonyl levels and restored GSH level in brain ($P < 0.05$). DG treatments lowered brain activity of GPX, SOD, and catalase (Table 8, $P < 0.05$); however, the intake of test compounds significantly retained brain GPX, SOD, and catalase activities ($P < 0.05$).

DISCUSSION

As reported by others[11,12] and our present study, DG treatments caused AD-like pathological characteristics such as increased Aβ deposition, AGEs generation, and oxidative stress. Our present study further found that the intake of SAC, SEC, and SPC down-regulated APP mRNA expression, diminished activity and expression of BACE1, retained PKC activity and expression, and decreased Aβ formation. In addition, we notified that these compounds markedly reduced the production of CML, pentosidine, ROS, protein carbonyl, and declined AR activity, as well as

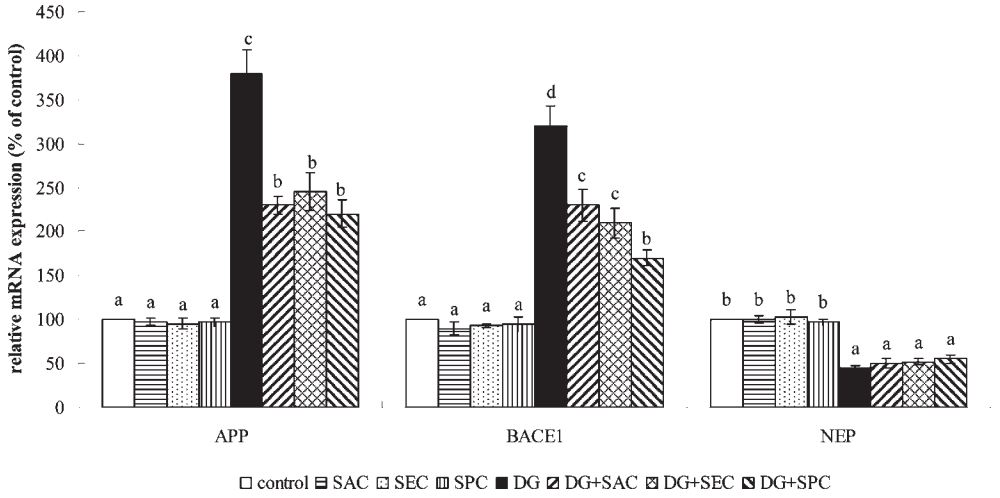

Figure 1. mRNA expression of APP, BACE1, and NEP in brain from mice with or without DG treatment and consumed 0 (control) or 1 g/L SAC, SEC, or SPC. Data are means ± SDs (n = 10). [a−d]Means among bars without a common letter differ; $P < 0.05$.

Table 4. Activity (nmol/mg Protein) of BACE1 and NEP in Brain from Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC[a]

	BACE1	NEP
control	7.62 ± 0.61 a	2.50 ± 0.24 a
SAC	7.34 ± 0.45 a	2.61 ± 0.19 a
SEC	7.28 ± 0.52 a	2.48 ± 0.22 a
SPC	7.53 ± 0.38 a	2.53 ± 0.27 a
DG	13.70 ± 0.89 d	1.03 ± 0.11 b
DG + SAC	11.43 ± 0.72 c	1.17 ± 0.09 b
DG + SEC	11.21 ± 0.68 c	1.14 ± 0.15 b
DG + SPC	9.36 ± 0.70 b	1.12 ± 0.16 b

[a] Data are means ± SD (n = 10). Means in a column without a common letter differ; $P < 0.05$.

maintained activity of GPX and SOD, which contributed to attenuate glycative and oxidative stress in brain of DG-treated mice. These novel findings suggested that these cysteine-containing compounds were able to penetrate the blood−brain barrier and exhibited anti-Aβ, antiglycative, and antioxidative neuroprotection to alleviate AD progression. Furthermore, these compounds might exert their functions at the level of transcription because they mediated mRNA expression of several factors.

Both APP level and BACE1 activity are crucial factors responsible for Aβ accumulation and AD progression.[18] So far, decreasing APP and/or inhibiting BACE1 have been considered as targets for developing therapeutic strategy for AD.[18,19] Aβ_{1-40} and Aβ_{1-42} are major Aβ forms that occur in the brain tissue and circulation of AD patients, and they also play clinical significance for AD deterioration because both are insoluble and able to cause aggregation of amyloid plaques.[20,21] In our present study, the intake of test compounds lowered BACE1 activity and repressed the mRNA expression of APP and BACE1, which subsequently decreased available Aβ precursors and lowered Aβ formation. Our data regarding brain levels of Aβ_{1-40} and Aβ_{1-42} also agreed that the supplementation of test compounds reduced Aβ production. These results indicated that these compounds exhibited anti-Aβ action via mediating APP and BACE1. On the other hand, Aβ could be degraded by a variety of proteases including NEP, which favors Aβ catabolism and clearance.[22] In our present study, treatments from cysteine-containing compounds failed to affect NEP expression and activity. Thus, the anti-Aβ effects of these agents was not due to they regulate this protease. It is reported that PKC activation lowered Aβ accumulation via a modulating nonamyloidogenic pathway of APP cleavage and led to the generation of soluble αAPP, which was released into the extracellular media, and precluded the deposition of Aβ.[23,24] Our results revealed that SAC, SEC, and SPC restored PKC activity and improved DG-caused down-regulation in PKC-α and PKC-γ expression in mice brain under AD-like conditions, which further benefited APP cleavage and decreased the available APP for Aβ formation. These findings suggested that the anti-Aβ action of these test compounds might be partially due to they enhance PKC activation.

Enhanced glycative stress is involved in AD progression. CML and pentosidine, two AGEs, have been implied in AD associated pathological development.[6,25] The lower generation of CML and pentosidine in brain tissue of test compound-treated mice as we observed indicated that glycative injury in those mice has been mitigated. AR and SDH are two key enzymes responsible for AGEs generation in the polyol pathway. Increased activity and expression of these enzymes facilitate the production of sorbitol and fructose, which in turn promote AGEs formation and glycative stress.[26,27] Our present study found that three test compounds declined both activity and mRNA expression of AR, which subsequently decreased the production of sorbitol. Although these test compounds failed to affect SDH activity and expression, it is highly possible that the lower available sorbitol further decreased fructose production. Because fructose and sorbitol levels had been reduced, the observed lower formation of CML and pentosidine could be partially explained. These findings indicated that these compounds could alleviate glycative stress in brain via suppression of the polyol pathway and inhibition of AGEs formation, which consequently contributed to attenuate AD progression.

Oxidative damage is another hallmark of AD.[28] It is reported that AD patients had increased carbonyl compounds and/or decreased GSH level in circulation.[29,30] In our present study, the intake of test compounds increased the GSH content in brain from mice without DG treatment. This finding implied that these

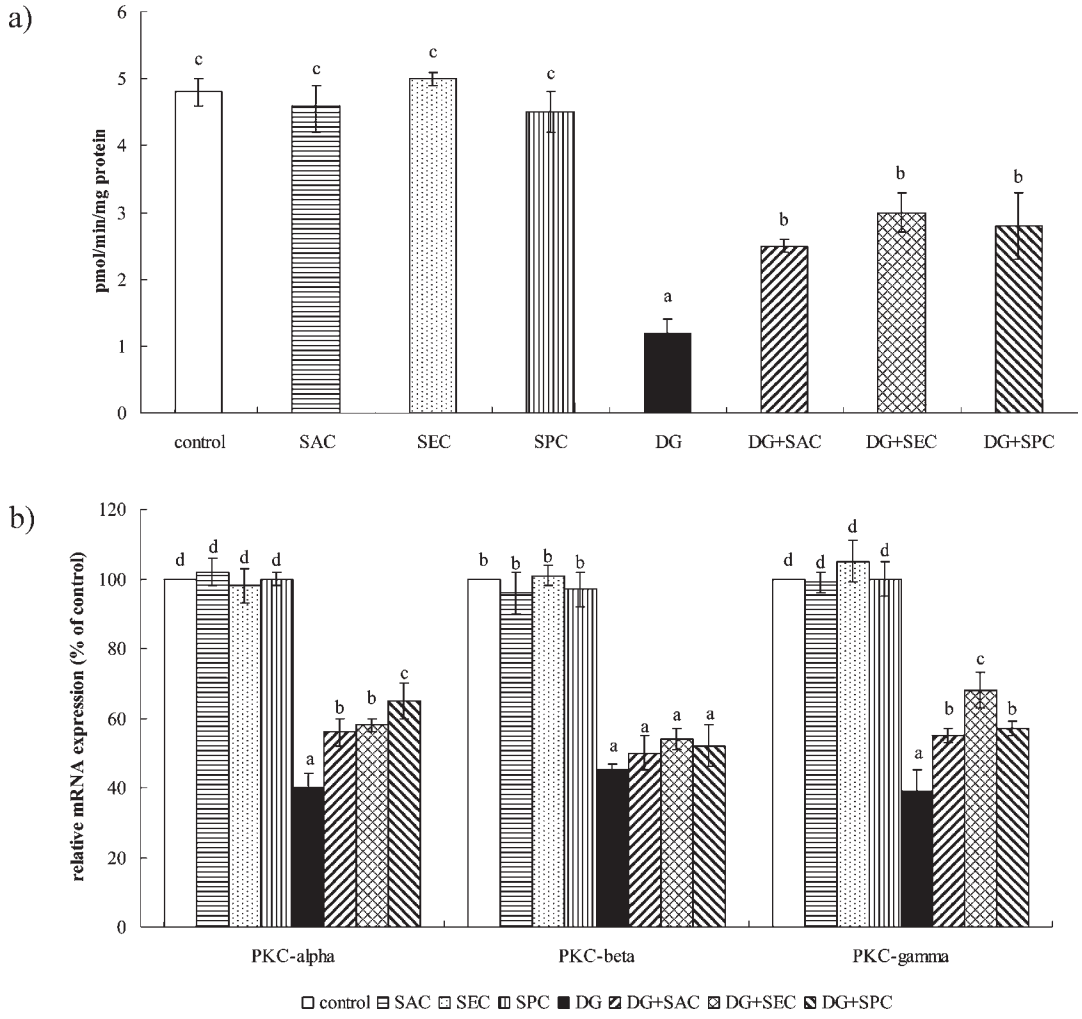

Figure 2. Activity (pmol/min/mg protein, upper part) and expression (lower part) of PKC in brain from mice with or without DG treatment and consumed 0 (control) or 1 g/L SAC, SEC, or SPC. Data are means ± SDs (n = 10). Means among bars without a common letter (a−d) differ; $P < 0.05$.

Table 5. Level of CML, Pentosidine, Sorbitol, and Fructose in Brain from Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC[a]

	pmol/mg protein		nmol/mg protein	
	CML	pentosidine	sorbitol	fructose
control	6 ± 3 a	0.20 ± 0.07 a	3.21 ± 0.31 a	13.0 ± 1.4 a
SAC	4 ± 2 a	0.17 ± 0.04 a	3.06 ± 0.24 a	12.8 ± 0.9 a
SEC	5 ± 3 a	0.19 ± 0.05 a	2.89 ± 0.19 a	12.7 ± 1.0 a
SPC	5 ± 4 a	0.18 ± 0.06 a	3.20 ± 0.26 a	12.8 ± 0.6 a
DG	64 ± 9 c	1.39 ± 0.12 c	8.75 ± 0.67 d	72.9 ± 5.8 c
DG + SAC	32 ± 5 b	0.67 ± 0.05 b	5.13 ± 0.21 b	30.1 ± 2.7 b
DG + SEC	30 ± 6 b	0.56 ± 0.07 b	6.09 ± 0.33 c	29.7 ± 2.2 b
DG + SPC	34 ± 7 b	0.71 ± 0.08 b	5.16 ± 0.38 b	31.0 ± 3.0 b

[a] Data are means ± SD (n = 10). Means in a column without a common letter differ; $P < 0.05$.

Table 6. Activity of AR (nmol/min/mg Protein) and SDH (U/g Protein) in Brain from Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC[a]

	AR	SDH
control	1.07 ± 0.25 a	4.11 ± 0.61 a
SAC	0.98 ± 0.16 a	4.14 ± 0.57 a
SEC	1.03 ± 0.19 a	3.96 ± 0.45 a
SPC	1.05 ± 0.08 a	4.02 ± 0.64 a
DG	3.82 ± 0.30 d	7.81 ± 1.02 b
DG + SAC	2.40 ± 0.24 c	7.12 ± 0.84 b
DG + SEC	1.68 ± 0.16 b	7.20 ± 0.69 b
DG + SPC	2.29 ± 0.19 c	7.27 ± 0.93 b

[a] Data are means ± SDs (n = 10). Means in a column without a common letter differ; $P < 0.05$.

agents might be able to spare GSH and favor GSH homeostasis, which definitely contributed to enhance antioxidative protection for this tissue. In addition, we found that the supplement of these compounds not only maintained GSH level but also effectively restored GPX, catalase, and SOD activities in brain from DG-treated mice. These results explained the lower formation of MDA, ROS, and protein carbonyl and indicated that SAC, SEC, and SPC could abate oxidative injury in brain of DG-treated mice via both nonenzymatic and enzymatic antioxidant protective

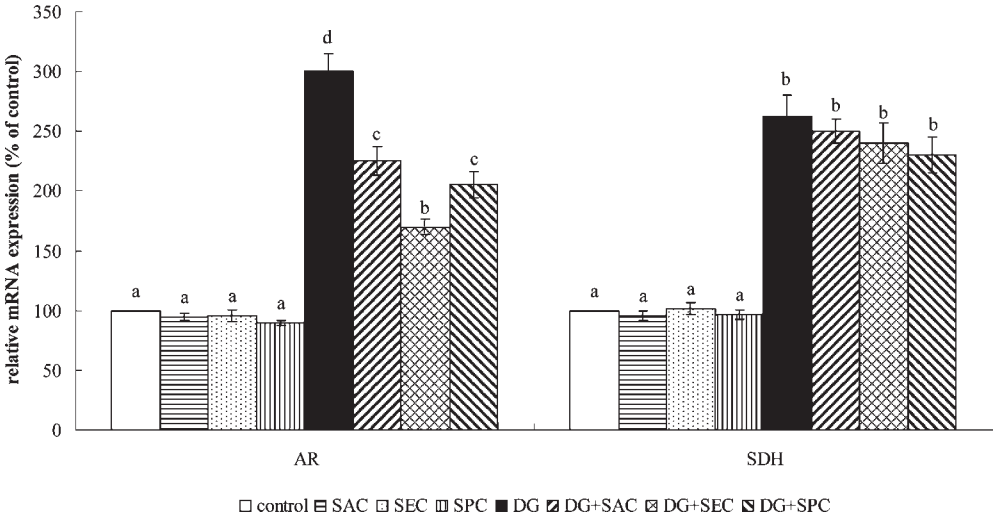

Figure 3. Expression of AR and SDH in brain from mice with or without DG treatment and consumed 0 (control) or 1 g/L SAC, SEC, or SPC. Data are means ± SDs (n = 10). Means among bars without a common letter (a−d) differ; $P < 0.05$.

Table 7. Level of MDA, ROS, Protein Carbonyl, and GSH in Brain from Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC[a]

	MDA (μmol/mg protein)	ROS (nmol/mg protein)	protein carbonyl (pmol/mg protein)	GSH (ng/mg protein)
control	0.26 ± 0.10 a	0.24 ± 0.07 a	16.8 ± 1.2 a	94 ± 8 c
SAC	0.21 ± 0.08 a	0.17 ± 0.09 a	14.1 ± 1.3 a	130 ± 12 d
SEC	0.20 ± 0.06 a	0.18 ± 0.05 a	15.2 ± 0.9 a	126 ± 15 d
SPC	0.18 ± 0.05 a	0.17 ± 0.04 a	14.5 ± 1.0 a	125 ± 10 d
DG	1.51 ± 0.33 c	1.37 ± 0.23 c	141.9 ± 7.3 c	46 ± 5 a
DG + SAC	1.02 ± 0.25 b	0.94 ± 0.16 b	80.1 ± 4.7 b	97 ± 7 c
DG + SEC	1.05 ± 0.17 b	1.03 ± 0.13 b	84.2 ± 5.0 b	79 ± 6 b
DG + SPC	0.92 ± 0.06 b	0.87 ± 0.10 b	82.1 ± 5.2 b	77 ± 6 b

[a] Data are means ± SDs (n = 10). Means in a column without a common letter differ; $P < 0.05$.

Table 8. Activity (U/mg Protein) of GPX, SOD, and Catalase in Brain from Mice with or without DG Treatment and Consumed 0 (Control) or 1 g/L SAC, SEC, or SPC[a]

	GPX	SOD	catalase
control	22.0 ± 2.3 d	7.3 ± 1.5 c	2.7 ± 0.6 c
SAC	24.1 ± 2.6 d	7.5 ± 1.7 c	2.6 ± 0.8 c
SEC	23.5 ± 2.0 d	7.7 ± 1.2 c	2.9 ± 0.4 c
SPC	22.9 ± 2.2 d	7.8 ± 1.4 c	2.3 ± 0.7 c
DG	8.8 ± 1.0 a	1.9 ± 0.7 a	0.6 ± 0.2 a
DG + SAC	16.2 ± 1.6 c	3.7 ± 1.0 b	1.2 ± 0.5 b
DG + SEC	12.9 ± 1.4 b	4.0 ± 0.9 b	1.4 ± 0.4 b
DG + SPC	13.3 ± 1.5 b	4.2 ± 1.1 b	1.5 ± 0.3 b

[a] Data are means ± SDs (n = 10). Means in a column without a common letter differ; $P < 0.05$.

actions. It is reported that Aβ deposition favors free radicals generation and exacerbates oxidative damage.[31] In our present study, test compounds markedly reduced Aβ production in brain, which might in turn benefit lowering free radicals formation and mitigating oxidative stress. On the other hand, it is known that free radicals could promote AGEs formation.[32] Thus, it is highly possible that the lower AGEs production in brain from test compound-treated mice as we observed was partially due to these compounds diminishing free radicals generation via their antioxidative activities.

It has been documented that AD could be considered as type 3 diabetes mellitus (DM) because both DM and AD shared common pathological features including oxidative and glycative stress.[33,34] The results of our present study revealed that AD progression not only increased the production of AGEs but also enhanced the activity and expression of glycation-associated enzyme such as AR in brain. These findings supported that there was a closed link between AD and DM and agreed that AD was a form of DM. Thus, the agents with anti-AD activity might also benefit from the prevention or treatment of DM. It is interesting to find that the neuroprotective effects from three test agents in DG-treated mice were different because SAC was more effective in maintaining GPX activity, SEC was marked in restoring PKC-γ expression, and SPC was greater than other agents in lowering Aβ_{1-40} generation and suppressing BACE1 expression and activity. Obviously, the allyl group of SAC, ethyl group of SEC, and propyl group of SPC played important roles in determining their bioactivities. These compounds are hydrophilic peptide derivates and naturally formed in *Allium* foods such as garlic. Further studies are necessary to ensure the safety of these agents before they are applied for humans. It is known that the content of these cysteine-containing compounds in *Allium* plants is

dependent on the species or vegetation period.[35] Thus, it may not be always possible to obtain these compounds by supplementing the diet with garlic or other *Allium* plants.

In conclusion, the treatments from SAC, SEC, or SPC effectively decreased Aβ production and alleviated glycative and oxidative stress in DG-treated mice. These agents exhibited anti-Aβ, antiglycative, and antioxidative effects by suppressing APP and BACE1 expression, retaining activity and expression of PKC, declining activity and expression of AR, and enhancing the activity of GPX, SOD, and catalase. These findings support that these compounds were potent agents against the progression of neurodegenerative disorders such as AD.

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