JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Caffeoylquinic Acid-Rich Purple Sweet Potato Extract, with or without Anthocyanin, Imparts Neuroprotection and Contributes to the Improvement of Spatial Learning and Memory of SAMP8 Mouse

Kazunori Sasaki,[†] Junkyu Han,[‡,§] Hidetoshi Shimozono,[∥] Myra O. Villareal,[§] and Hiroko Isoda*[,‡,§]

[†]Graduate School of Life and Environmental Sciences, [‡]Faculty of Life and Environmental Sciences, and [§]Alliance for Research on North Africa (ARENA), University of Tsukuba, 1-1-1 Tennodai, Tsukuba City, Ibaraki 305-8572, Japan

[∥]Kagoshima Prefectural Institute of Food Processing and Utilization, 2200 Kinpouchou, Oono, Minamisatsuma City, Kagoshima, 899-3401, Japan

ABSTRACT: The effects of caffeoylquinic acid (CQA)-rich purple sweet potato (PSP) extract, with (PSPEa) or without (PSPEb) anthocyanin, on the improvement of spatial learning and memory of senescence-accelerated prone mouse strain (SAMP) 8 was determined. SAMP8 was treated with 20 mg/kg/day of PSPEa or PSPEb for 30 days. The effect on spatial learning and memory and the molecular mechanism of this effect were determined in vivo (SAMP8) and in vitro (SH-SY5Y cells). PSPEa or PSPEb reduced the escape latency time of SAMP8 by 17.0 ± 8.0 and 14.2 ± 5.8 s ($P < 0.01$), respectively. PSPEa administration induced an overexpression of antioxidant-, energy metabolism-, and neuronal plasticity-related proteins in the brain of SAMP8. Additionally, PSPEa and PSPEb increased the cell viability by 141.6 and 133% as compared to $A\beta_{1-42}$-treated cells. These findings suggest that PSP rich in CQA derivatives with or without anthocyanidine had a neuroprotective effect on mouse brain and can improve the spatial learning and memory of SAMP8.

KEYWORDS: *purple sweet potato, caffeoylquinic acid, senescence-accelerated prone mouse strain 8, spatial learning and memory, Morris water maze test*

INTRODUCTION

Sweet potato (*Ipomoea batatas*) is the seventh most important food crop in many Asian and African countries, and in Japan alone, it was estimated in 2005 that one person consumes about 6.8 kg of sweet potato (FAOSTAT 2009 at http://faostat.fao.org). Most sweet potato cultivars have white or yellow flesh, but there are also some cultivars with orange flesh that have high carotenoid contents or with purple flesh that have high anthocyanin contents. In addition, the nutritional and health-promoting benefits of the consumption of purple sweet potato (PSP) have recently gained popularity,[1] because it is a rich source of a large number of diverse nutrients, such as vitamins, minerals, and polyphenols, giving it an especially high antioxidative potential.[2] Also, PSP contains phenolic compounds such as anthocyanins and caffeoylquinic acids (CQA). Anthocyanins have been reported to exhibit several physiological functions such as antioxidative effects,[3,4] a neuroprotective effect, and the ability to decrease amyloid-β (Aβ) peptide-mediated cytotoxicity in SH-SY5Y neurocytes.[5] PSP has a higher amount of CQA derivatives (137 ± 37.5 mg/100 g) than common sweet potato (25.7 ± 4.3 mg/100 g),[6] making it one of the suitable dietary sources of CQA. The presence of CQAs in PSP therefore makes it more nutritious and more beneficial to health than most foods. CQA derivatives have neuroprotective effects on hydrogen peroxide- and Aβ-induced cell death in SH-SY5Y cells.[7] In our previous study, di-CQA, one of the CQA derivatives, has shown a neuroprotective effect against Aβ-induced cell death of human neuroblastoma SH-SY5Y. Moreover, oral administration of di-CQA to senescence-accelerated prone mouse (SAMP) 8 has significantly improved spatial learning and memory, and it was observed that the mouse brain had an overexpression of phosphoglycerate kinase-1, one of the enzymes in the glycolysis pathway.[8]

There are approximately 35.6 million people worldwide with dementia, and this number is expected to increase to over 115 million by 2050. Alzheimer's disease (AD), the most common neurodegenerative disorder, is a progressive, age-dependent neurodegenerative disorder of the cortex and hippocampus, eventually leading to the impairment of the cognitive function of the brain. The presence of intracellular neurofibrillary tangles and extracellular Aβ plaques in these learning and memory regions are the hallmarks of AD.[9] Free radicals produced during oxidative stress are speculated to be pathologically important in AD and other neurodegenerative diseases.[10] It is likely that the neurotoxicity induced by Aβ is caused by oxidative stress and that this Aβ-mediated oxidative stress is involved in the pathogenesis of AD. The free radical theory supports the hypothesis that aging occurs when there is an accumulation of oxidative damage, DNA mutation, and lipid and protein dysfunction. Recently, many important and naturally occurring flavonols and flavones have been shown to have a pharmacological benefit for AD.[11] Likewise, dietary polyphenols may be helpful in preventing or delaying such a disease.

Therefore, PSP, with both anthocyanins and CQAs, will be very useful for protecting cells against neurodegenerative

Received: September 26, 2012
Revised: May 7, 2013
Accepted: May 7, 2013
Published: May 7, 2013

dx.doi.org/10.1021/jf3041484 | *J. Agric. Food Chem.* 2013, 61, 5037−5045

disorders such as AD. Previous reports on the effect of PSP on the improvement of spatial learning and memory focused on the effect of anthocyanins[12,13] and not on CQA. In the present study, two kinds of PSP extracts, with (PSPEa) or without (PSPEb) anthocyanins, were used to determine the effect of CQA alone. Both CQA and anthocyanins have shown a neuroprotective effect and have caused an in improvement in spatial learning and memory;[12,13] we want to find out the effect of CQA alone or with anthocyanins because it is also possible that there is a synergistic effect between CQA and anthocyanins. The present study was conducted to determine the effect of PSP on spatial learning and memory of SAMP8 mice and to investigate the mechanism underlying this effect. In addition, we have previously reported that CQA can help improve the spatial learning and memory;[8] here, we determined the molecular mechanism behind the neuroprotective effect of CQA that imparts spatial learning and memory of SAMP8 mouse.

MATERIALS AND METHODS

Chemicals. $A\beta_{1-42}$ was purchased from Funakoshi (Japan). Coomassie Tablets, PhastGel R-350, and a 2-D quant kit were from GE Healthcare (Japan). Dulbecco's modified Eagle's medium (DMEM), Ham's F-12, and fetal bovine serum (FBS) were from Sigma Aldrich Co., Ltd. (Irvine, United Kingdom). Penicillin-streptomycin was from Lonza Inc. (Walkersville, MD), and OPTI-MEM was purchased from Gibco (Japan). NH_4HCO_3 was from Sigma (United States). Acetonitrile (ACN) (HPLC grade) and formic acid were from Wako (Japan). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was from (Dojindo, Japan). An adenosine triphosphate (ATP) bioluminescence assay kit was from TOYO Ink (Japan).

Sample Preparation. The Japanese PSP variety "Ayamurasaki", used in this study, was obtained from Tanegashima Region (Japan), and the contents were 53.3, 24.0, 19.5, and 10.7 mg/100 g 5-CQA, 3,4-di-CQA, 3,5-di-CQA, and 4,5-di-CQA, respectively (dry weight), as we have previously reported.[14] The composition of PSP, as previously reported, is summarized in Table 1.[6,14] Whole PSPEa and PSP extract subjected to an ethyl acetate liquid/liquid extraction method[15] to remove the anthocyanins (PSPEb) were used in this study. PSPEb treatment will show the effects of CQAs alone. To prepare the extract, PSP was washed, cut, and placed inside the compression machine to concentrate the extract and was filtered prior to use (PSPEa). To prepare PSP without anthocyanins, PSPEa was filtered, steamed for 40 min, minced, and then dried using a double drum type drier. The dried PSPEb was then crushed in 80% ethanol, filtered, and concentrated using an evaporator. The PSPEb was then divided into fractions containing phenolic compounds or anthocyanins, using the ethyl acetate liquid/liquid extraction method. The phenolic fraction was used to see the effect of PSP without the anthocyanins.

Table 1. Composition of PSP[a]

component	amount	source
sucrose	3.08 ± 0.09 g/100 g	ref 6
fructose	0.39 ± 0.03 g/100 g	
glucose	0.50 ± 0.04 g/100 g	
total vitamin C	23.6 ± 0.9 mg/100 g	
anthocyanin (C3G equivalent volume)	258 ± 17.3 mg/100 g	
polyphenol (CQA equivalent volume)	137 ± 37.5 mg/100 g	
5-CQA	53.3 mg/100 g	ref 14
3,4-di-CQA	24.0 mg/100 g	
3,5-di-CQA	19.5 mg/100 g	
4,5-di-CQA	10.7 mg/100 g	
total amount of CQA	108 mg/100 g	

[a]Abbreviation: C3G, cyanidin-3-glucoside.

Animals and Sample Treatment. The 4 month old male SAMP8 and senescence-accelerated resistant mice (SAMR) 1 used in the experiment were obtained from Japan SLC, Inc. Mice were housed one mouse per cage and were allowed access to water and food ad libitum, under a 12/12 h light/dark cycle. The protocol for this animal experiment was approved by the Animal Care and Use Committee of the University of Tsukuba. After 14 days of acclimatization to the laboratory conditions, the mice were divided into five groups: SAMR1 control group (n = 5), SAMR1 PSPEa-administered group (n = 5), SAMP8 control group (n = 5), SAMP8 PSPEa-administered group (n = 5), and PSPEb-administered group (n = 5). The PSPEa-administered group and PSPEb-administered group were fed with PSPEa or PSPEb mixed with tap water (20 mg/kg day) for 30 days using an oral administration tube and syringe. Control groups were administered an equivalent volume of tap water.

Morris Water Maze (MWM) Test. The MWM test was carried out using a circular pool (120 cm in diameter and 45 cm in height) with a featureless inner surface. In this experiment, four symbols (a triangle, a circle, a cross mark, and a square) were attached on the inner surface of the circular pool. The pool was filled to a depth of 30 cm with water (23 ± 2 °C) and divided into four quadrants designated as north, east, west, and south. A platform (10 cm in diameter) was placed in the northeast quadrant and submerged 1 cm below the water surface so that it was invisible at water level. The mice were then given a trial session four times each day for 1 week on a platform fixed in the same location in the pool for the whole duration of the experiment. Each mouse was placed on the platform for 30 s every day for 7 days. During the trial period, PSPEa and PSPEb were not administered to the mice.

Tissue Preparation and Proteomics Analysis Using Two-Dimensional Gel Electrophoresis (2-DE). Mice were sacrificed by dislocation of the cervical spine on the day of the last trial for the MWM test. The brain samples were rapidly removed from the sacrificed mouse, quickly dissected in liquid nitrogen, and stored in −80 °C until use. For 2-DE, tissue samples were washed with PBS, mechanically homogenized in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, and 40 mM Tris, pH 8.0, 1 mL per 0.1 g tissue), and centrifuged (15000g, 10 min, 4 °C). The pellet was then discarded, and the concentration of the protein in the supernatant was estimated using the 2-D quant kit. 2-DE (Amersham Biosciences, Sweden) was performed using 180 mm pH 3−10 immobilized pH gradients (IPG) strips. The protein sample (350 μg) was loaded on IPG strips that were actively rehydrated at 20 °C for 12 h. The isoelectric focusing of strips loaded was done at 20 °C and was run as follows: 300 V for 3 h, 600 V for 3 h, 1000 V for 3 h, and 8000 V for 4 h. IPG strips were then equilibrated for 20 min prior to second-dimension separation in 6 M urea, 50 mM Tris-HCl, pH, 30% glycerol, 2% sodium dodecyl sulfate (SDS), and 0.25% DTT, followed by re-equilibration for 20 min in the same buffer containing 4.5% iodoacetamide in place of DTT. When the equilibration was finished, the strips were loaded onto 12.5% acrylamide vertical gels, and the separation of proteins with different molecular weight was carried out at 1 W per gel for 1 h, followed by 2 W per gel for 16 h.

Coomassie brilliant blue staining using Coomassie Tablets, PhastGel R-350, was performed according to the manufacturer's instructions. The gels were subjected to image analysis using ImageMaster 2D Platinum software (version 4.9; GE Healthcare, Japan). The average mode of background subtraction was used to normalize the intensity value, which represents the amount of protein per spot. After spot detection and matching, the spots of interest were manually selected, and the relative intensities were obtained. Spot intensities were expressed as percentages (% vol) of relative spot volumes with the value (or OD) of each pixel integrated in the spot intensity.

In-Gel Digestion and Mass Spectrometry. The protein spots in the gel were excised using a picking tip (HI-TECH CO., Ltd., Japan) and transferred into 1.5 mL eppendorf tubes. The protein spots were decolorized with 100 μL of 50% acetonitrile (ABI: P/N400313 solvent

B)/25 mM NH_4HCO_3. After decolorization, the spots were dehydrated with 100 μL of 100% ACN and dried using SpeedVac (SCRUM Inc., Japan). The addition of 100 μL of 10 mM DTT/25 mM NH_4HCO_3 to the protein spots was done and shaken for 1 h. Protein samples spots were then washed with 25 mM NH_4HCO_3 (washing solution) for 10 min. Protein spots were alkylated with 100 μL of 55 mM iodoacetamide/25 mM NH_4HCO_3 for 45 min in dark. After alkylation, protein spots were washed with 100 μL of washing solution for 10 min. Finally, protein spots were digested with 20 μL of trypsin [(10 μg/mL Modified Trypsin Sequencing grade (Promega, United States), 50 mM NH_4HCO_3)], and the peptides were extracted with 50% ACN/5% formic acid. The peptide solution was concentrated using a SpeedVac until the volume was only about 5−10 μL and analyzed using LC/MS/MS 3200Q trap system. The mass spectra of the identified proteins were matched to the mass spectra of protein in NCBI protein databases to identify the protein.

Cell Culture. The human neuroblastoma SH-SY5Y cell line was obtained from American Type Culture Collection. SH-SY5Y neuroblastoma cells were cultured in 100 mm Petri dish or in 96-well plates with a 1:1 (v/v) mixture of DMEM and Ham's F-12 nutrient mixture supplemented with 15% FBS and 1% penicillin (5000 μg/mL)−streptomycin (5000 IU/mL) solution at 37 °C in a 95% humidified air−5% CO_2 incubator. A serum-free Eagle's minimum essential medium (OPTI-MEM) was used to culture the cells for the cell viability assay, intracellular ATP measurement, and intracellular reactive oxygen species (ROS) measurement. The PSPEa and PSPEb used contained 1.55 and 5 μM di-CQA, respectively, for all in vitro assays.

Determination of Cell Proliferation. Cell proliferation was measured using the MTT method. SH-SY5Y cells cultured in 96-well plate (fibronectin-coated plate) (BD BioCoat, United States) were treated with PSPEa or PSPEb and treated to 15 μM $A\beta_{1-42}$ for 72 h. After sample treatment, 100 μL of Opti-MEM and 10 μL of MTT (5 mg/mL) were added, and the cells were incubated further for 6 h. The MTT formazan formed was dissolved in 100 μL of 10% SDS (w/v), and the absorbance was measured using the microtiter plate reader (Dainippon Sumitomo Pharma Co., Ltd., Japan).

Measurement of the Intracellular ATP Production. The cellular ATP content was assessed using ATP bioluminescence assay kit. SH-SY5Y cells (2×10^5 cells/mL) were cultured in a 96-well plate (fibronectin-coated plate) and treated with PSPEa or PSPEb for 4, 8, and 16 h. After treatment with PSPEa or PSPEb, 100 μL of the luciferin-luciferase solution, which contains the cell lysis buffer and luciferase luminescence reagent, was added, and the bioluminescence was measured using a luminometer (Powerscan HT; Dainippon Sumitomo Pharma Co, Ltd., Japan).

Metabolomics Analysis. SH-SY5Y cells (2×10^5 cells/mL) were treated with PSPEa and subjected to metabolites extraction by adding 1 mL of methanol that contained 10 μM internal standard solution [20 μmol/L each of methionine sulfone and 2-(*N*-morpholino)-ethanesulfonic acid], which were used for identification of metabolites using mass spectrometry. The addition of 1 mL of chloroform and 400 μL of Milli-Q water followed, and the samples were centrifuged at 2300*g* at 4 °C for 5 min. After centrifugation, the aqueous phase was transferred to a filtration tube (Millipore, United States) and centrifuged at 9100*g* at 4 °C for 120 min. Filtrates were dried and dissolved in 50 μL of Milli-Q water prior to analysis. Metabolomics analysis was performed using capillary electrophoresis time-of-flight mass spectrometry (CE-ToFMS) (Agilent Technologies, Inc., United States).

Measurement of the Intracellular ROS. SH-SY5Y cells (2×10^5 cells/mL) were seeded in fibronectin-coated 96-well plates and were incubated in Opti-MEM with increasing concentrations of $A\beta_{1-42}$ with or without PSPEa or PSPEb for 1 h. The cells were then incubated with 10 μM dichlorodihydrofluoresein diacetate (DCFH-DA) at 37 °C for 1 h and washed with PBS, and the fluorescence intensity of dichlorofluoresein (DCF) was measured using a microtiter plate (Dainippon Sumitomo Pharma Co., Ltd., Japan) reader at an excitation wavelength of 485 nm and emission wavelength of 528 nm. Intracellular ROS was monitored using the fluorescent probe DCFH-DA.[16] Intracellular H_2O_2 or low-molecular weight peroxides oxidize DCFH-DA to the highly fluorescent compound DCF.

Statistical Analysis. All of the results were expressed as means ± standard error deviations (SDs), and the statistical evaluation was performed using Student's *t* test when two value sets were compared. $P < 0.05$ was considered to be statistically significant.

RESULTS

PSPEa and PSPEb Improved the Spatial Learning and Memory of SAMP8. To evaluate the effect of PSPEa and PSPEb on the spatial learning and memory in SAMP8 mice, a MWM test was employed. The MWM test is a widely used tool to assess spatial learning and memory in rodents. In the MWM test, the animal is placed on the platform of the pool concealed under the surface of the water. The swimming time to reach the platform was measured to assess the effect of PSPEa or PSPEb on spatial learning and memory. The amount of time (in seconds) spent to reach the platform indicates how quickly it can learn and memorize the location of the platform. Shorter swimming times as compared to the untreated control indicates an improvement in spatial learning and memory. As shown in Figure 1A, the escape latency time of SAMP8 administered with PSPEa group ($n = 5$) was significantly decreased as compared to the SAMP8 control group after 3 days of training. In addition, the escape latency time of PSPEb-administered SAMP8 groups was also significantly decreased as compared to SAMP8 control groups after 3 days (Figure 1A). At the best, PSPEa and PSPEb decreased the escape latency time of SAMP8 by 17.0 ± 8.0 and 14.2 ± 5.8 s ($P < 0.01$). However, the escape latency time of PSPEb-administered group was increased at 7 days after training. On the other hand, there was no observed difference between the escape latency time of SAMR1 control groups ($n = 10$) and SAMR1 groups administered with PSPEa, suggesting that PSP is safe for consumption or as food (Figure 1B). However, the escape latency time of SAMR1 control and PSPEa groups ($n = 10$) was higher as compared to SAMR1 groups and SAMP8 administered with PSPEb ($P < 0.01$) at the seventh day of the experiment (Figure 1A).

PSPEa Induced the Overexpression of Antioxidation-, Energy Metabolism-, and Neuronal Plasticity-Related Proteins in Mouse Brain. The results of the MWM test revealed that PSPEa and PSPEb can improve the spatial learning and memory of SAMP8. However, at day 7 of training, the escape latency time of PSPEb was increased (Figure 1A). Therefore, the effect of PSPEa on the improvement of spatial learning and memory was greater than PSPEb and was subjected to 2D electrophoresis, and the differentially expressed proteins were identified. To determine the molecular mechanism of the effect of PSPEa on the protein expression in mouse on the spatial learning and memory of SAMP8 mice administered with PSPEa, we analyzed the protein expression in SAMP8 brain. We found that the expressions of nine proteins were increased in SAMP8 mouse treated with PSPEa. Figure 2A−C presents the representative gel of the protein from whole brain homogenate of PSPEa-administered SAMP8 mice and SAMP8 control mice. The identified proteins (Tables 2 and 3) appeared in the expected molecular weights and pI ranges on the gels. The expression levels of all proteins were significantly increased in the brain of SAMP8 mice administered with PSPEa. Subjecting the spots to identification, we found that the expression of peroxiredoxin-2 (PRDX2), the protein associated with the antioxidative properties, was increased by administered with PSPEa. Also, the expressions

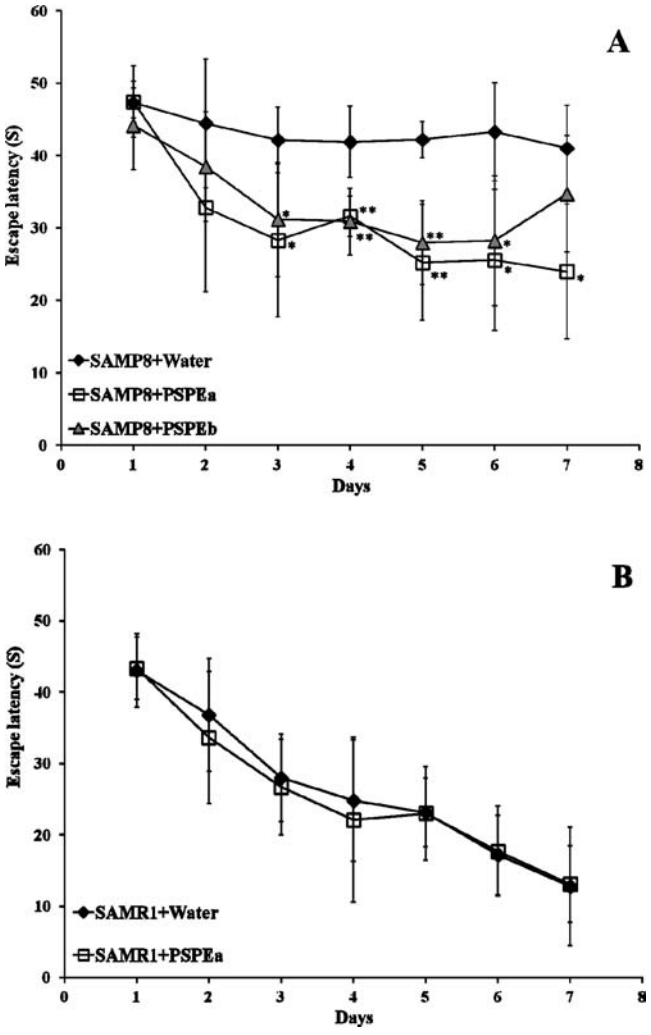

Figure 1. Effect of PSP extract, with (PSPEa) or without anthocyanin (PSPEb), on the spatial learning and memory of SAMP8 mice. SAMP8 water-treated group, SAMP8 PSPEa-treated group, and SAMP8 PSPEb-treated group (A). SAMR1 mice water-treated group and SAMR1 PSPEa-treated group (B). Each bar represents the mean ± SD (n = 5); *P < 0.05, **P < 0.01 vs SAMP8 water-treated group.

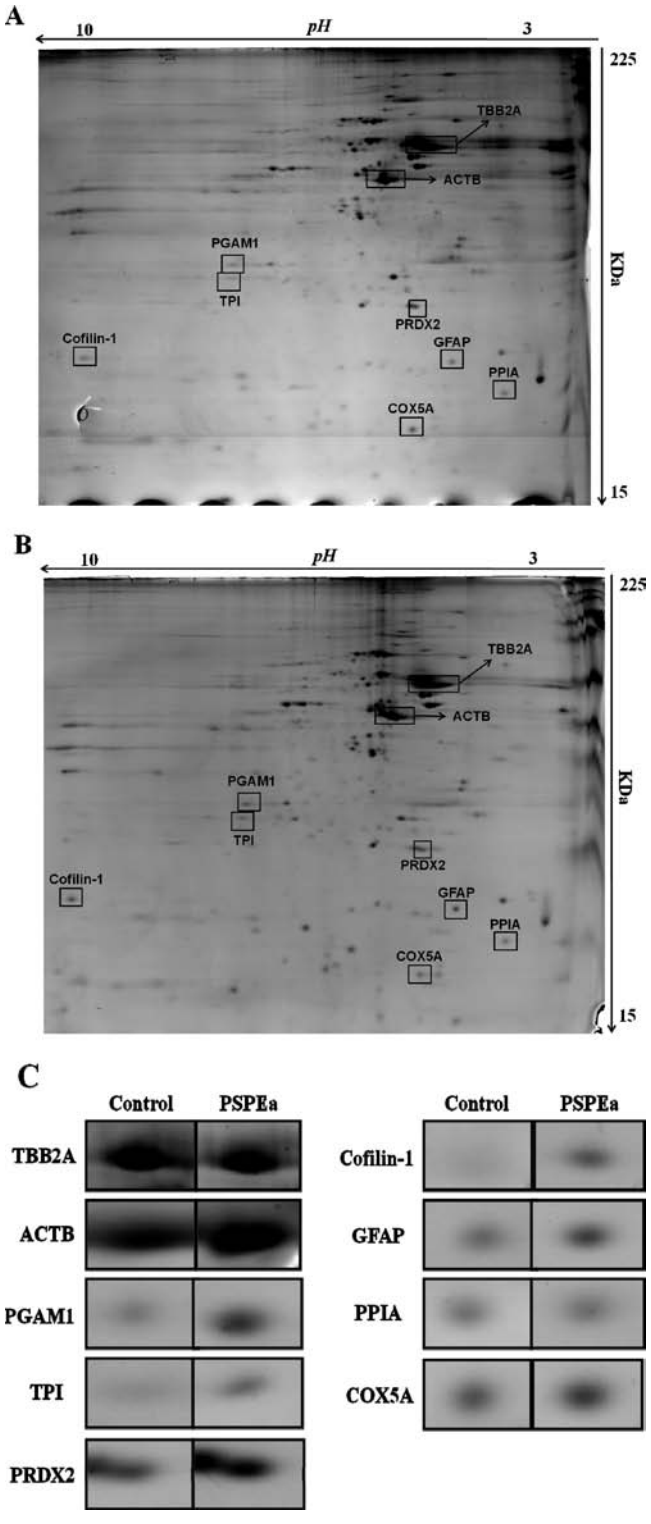

Figure 2. Two-dimensional gel electrophoresis gel of the brain of SAMP8 mice treated with water (A) and PSP (B) and magnified images of the spot (C).

of cytochrome oxidase subunit 5A (COX5A), triosephosphate isomerase (TPI), and phosphoglycerate mutase 1 (PGAM1), which are enzymes associated with energy metabolism, were increased. In addition, the expressions of peptidyl-prolyl cis/trans isomerase A (PPIA), a protein related to the neuronal cytoskeleton, glial fibrillary acidic protein (GFAP), the main intermediate filament of a mature astrocyte, tubulin-β 2A (TBB2A), actin-β (ACTB), and cofilin-1, a protein related to the cytoskeleton, were increased. Thus, treatment with PSPEa induced an increase in the expressions of antioxidant-, energy metabolism-, and neuronal plasticity-related proteins.

PSP Extract Inhibited Neuronal Cytotoxicity and Increased the Proliferation of SH-SY5Y Cells. The MTT assay was used to determine the effect of PSPEa and PSPEb on SH-SY5Y cell proliferation and on neuronal cytotoxicity. The results showed that exposure to Aβ_{1-42} resulted in a significant decrease in cell proliferation (Figure 3). The cell viability of Aβ_{1-42}-treated cells was significantly decreased to 56 ± 1.7% as compared to nontreated cells (P < 0.01). However, incubation with PSPEa reversed the Aβ_{1-42}-induced cell death, and the cell viability was significantly increased by 141.6 ± 2.3% as compared to the 100% of the Aβ_{1-42}-treated group (P < 0.01). PSPEb also prevented Aβ_{1-42}-induced cell death and improved the cell viability (133 ± 2.0%) as compared to the 100% of the Aβ_{1-42}-treated group (P < 0.01). Interestingly, the cell viability of cells treated with PSPEa and PSPEb, without Aβ_{1-42} treatment, was significantly increased (134.4 ± 3.3%) (129 ± 2.2%) as compared to the 100% of the untreated cells

dx.doi.org/10.1021/jf3041484 | J. Agric. Food Chem. 2013, 61, 5037−5045

Table 2. List of Identified Proteins in the Brain of SAMP8 Mice[a]

accession no.	identified protein	MW (kDa)	pI	score	sequence coverage (%)
NP_033476	TBB2A	50.3	4.78	412	31
NP_031419	ACTB	42.1	5.29	332	25
NP_075907	PGAM1	29.0	6.67	214	24
NP_033441	TPI	27.0	6.9	103	8
NP_035693	PRDX2	21.9	5.2	220	35
NP_031713	cofilin-1	18.8	8.22	45	35
AAN87913	GFAP	49.9	5.27	31	2
NP_032933	PPIA	18.1	7.74	63	19
NP_031773	COX5A	16.3	6.08	58	10

[a]Sequence coverage extends the percentage of the matched peptides. A score greater than 29 is considered significant ($P < 0.05$). Abbreviations: MW, molecular weight; pI, isoelectric point.

Table 3. Classification of Identified Proteins and Expression of These Proteins in Brain of SAMP8 and SAMR1 Mice[a]

protein name	relative expression change (SAMR1 + PSPa vs SAMR1 + water)	relative expression change (SAMP8 + PSPa vs SAMP8 + water)	function
PRDX2	1.36	1.65	antioxidative effect
PGAM1	1.07	1.67	energy metabolism
TPI	1.44	1.38	
COX5A	0.99	1.27	
TBB2A	1.11	1.37	neuronal plasticity
ACTB	0.86	1.45	
cofilin-1	1.27	1.84	
GFAP	0.74	1.55	
PPIA	1.26	1.73	

[a]Spot intensities were expressed as percentages (% vol) of relative spot volumes with the value (or OD) of each pixel integrated in the spot intensity. The expressions of the protein spots of the brain of SAMR1-fed PSP extracts (PSPEa) and water groups and SAMP8-fed PSPEa and water groups were compared, respectively.

($P < 0.01$). Moreover, comparing PSPEa and PSPEb, the neuroprotective effect of PSPEa was higher than that of the effect of PSPEb on cells with or without Aβ_{1-42} ($P < 0.05$) pretreatment ($P < 0.01$).

PSPEa and PSPEb Promoted the Intracellular ATP Production in SH-SY5Y Cells. We observed an increase in the cell proliferation in PSPEa and PSPEb-treated SH-SY5Y and decided to determine the ATP content of the cells. ATP is a multifunctional nucleotide that is also regarded as the "energy currency" of the cell and is also widely used as a marker of cell proliferation, as it transports chemical energy within cells for metabolism. The intracellular ATP production level in the PSPE-treated SH-SY5Y cells was measured using a luciferase method. SH-SY5Y cells treated with the PSPEa have significantly higher ATP production (173.3 ± 4.0%) as compared to the untreated group 4 h of treatment ($P < 0.01$) (Figure 4). After 8 h of treatment, the amount of ATP in the PSPEa-treated group was significantly increased to 142.8 ± 1.2% as compared to the untreated group ($P < 0.01$). Moreover, the ATP production in the PSPEa-treated group was also significantly increased to 123.4 ± 1.9% as compared to the untreated group after 16 h of treatment ($P < 0.01$). On the other hand, as compared to the control group, the ATP

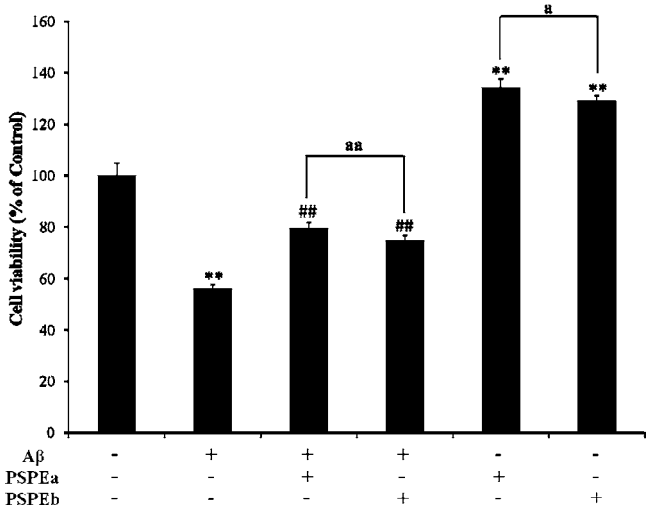

Figure 3. Effect of PSP extract, with (PSPEa) or without anthocyanin (PSPEb), on the viability of Aβ_{1-42}-treated SH-SY5Y cells. SH-SY5Y cells were treated with PSPEa or PSPEb or 15 μM Aβ_{1-42} for 72 h. The final concentrations of CQA in PSPEa and PSPEb were 1.55 and 5 μM, respectively. Each bar represents the mean ± SD ($n = 5$); $^{**}P < 0.01$ vs nontreated cells, $^{\#\#}P < 0.01$ vs Aβ-treated cells, and $P < 0.05$ (a) and $P < 0.01$ (aa) vs PSPEb-treated cells.

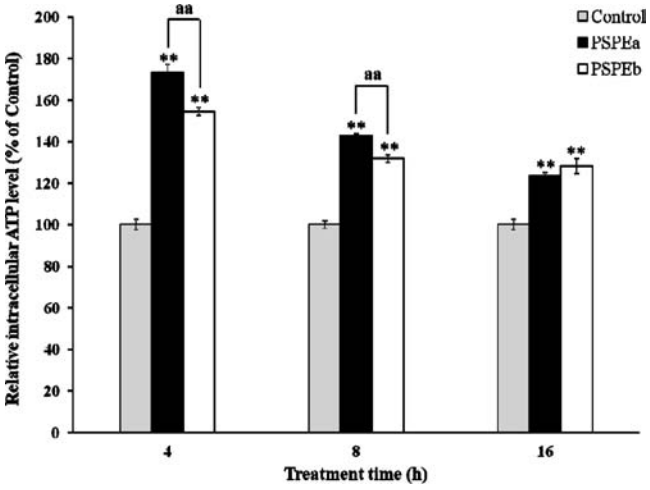

Figure 4. Effect of PSP extract, with (PSPEa) and without anthocyanin (PSPEb), on the intracellular ATP production of SH-SY5Y cells. SH-SY5Y cells were treated with PSPEa or PSPEb for 4, 8, and 16 h. The final concentrations of CQA contained in PSPEa and PSPEb were 1.55 and 5 μM, respectively. Each bar represents the mean ± SD ($n = 5$); $^{**}P < 0.01$ vs nontreated cells, aa $P < 0.05$ vs PSPEb-treated cells, and $P < 0.01$ (aa) vs PSPEb-treated cells.

produced by the PSPEb-treated group was also significantly increased to 154.5 ± 1.9, 131.9 ± 1.8, and 128.2 ± 3.5% after 4, 8, and 16 h of treatment ($P < 0.01$), respectively. Interestingly, comparing PSPEa and PSPEb treatment groups, the ATP production in the PSPEa-treated group was higher than that of the ATP production in the PSPEb-treated group at 4 and 8 h ($P < 0.01$).

PSPEa Promoted the Central Energy Metabolism in SH-SY5Y Cells. Because PSPEa was found to promote the intracellular ATP production, the mechanism for this effect was investigated by analyzing the expressions of the metabolites in the glycolysis and tricarboxylic acid (TCA) cycle by CE-ToFMS. In this study, 47 main metabolites were detected and

dx.doi.org/10.1021/jf3041484 | J. Agric. Food Chem. 2013, 61, 5037−5045

quantified (data not shown). It was noted that the concentrations of glycolysis metabolites glucose 6-phosphate (G6P), fructose 6-phosphate (F6P), fructose 1,6-diphosphate (F1,6P), 3-phosphoglyceric acid (3-PG), and phosphoenolpyruvic acid (PEP) were remarkably increased as compared to the PSPEa treatment group and the control group (Table 4). Moreover, TCA cycle metabolites such as acetyl CoA divalent (AcCoA), citric acid, *cis*-aconitic acid, and succinic acid were also increased in the PSPEa-treated group (Table 4).

Table 4. Quantification of Glycolysis and TCA Cycle Metabolites in PSP Extract (PSPEa)-Treated SH-SY5Y Cells[a]

metabolites	control (pmol/10^6 cells)	PSPEa (pmol/10^6 cells)	ratio (PSPEa/control)	function
G6P	300	480	1.6	glycolysis
F6P	70	112	1.6	
F1,6P	700	1260	1.8	
3-PG	200	320	1.6	
PEP	80	136	1.7	
AcCoA	15	36	2.4	TCA cycle
citric acid	5	6	1.2	
cis-aconitic acid	1300	1560	1.2	
succinic acid	900	1440	1.6	

[a]Metabolome analyses of nontreated cells and PSP extracts (PSPEa)-treated cells (1.55 μM di-CQA contained). The data of the table show amounts of metabolites (pmol/10^6 cells) determined by CE-TOFMS.

Intracellular ROS Production in SH-SY5Y Cells Was Inhibited by PSPEa and PSPEb. In this experiment, the degree of ROS accumulation after exposure to Aβ_{1-42} was measured to determine the effect of PSP on ROS production in cells pretreated with Aβ_{1-42}. As shown in Figure 5, treatment of SH-SY5Y cells with Aβ_{1-42} (5 μM) increased the DCF fluorescence in a concentration-dependent manner, the highest of which was 178.9 ± 20.4% as compared with the control.

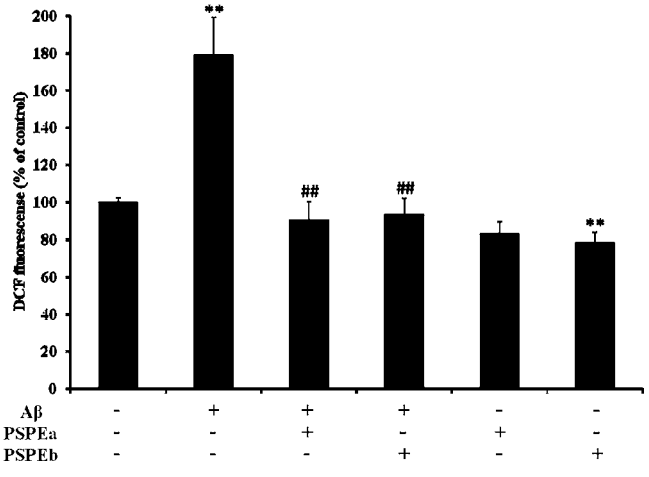

Figure 5. Effect of PSP extract, with (PSPEa) and without anthocyanin (PSPEb), on the intracellular ROS production of Aβ_{1-42}-treated SH-SY5Y cells. SH-SY5Y cells were treated with PSPEa or PSPEb or 5 μM Aβ_{1-42} for 72 h. The final concentrations of CQA contained in PSPEa and PSPEb were 1.55 and 5 μM, respectively. Each bar represents the mean ± SD (n = 5); $^{**}P < 0.01$ vs nontreated cells, and $^{\#\#}P < 0.01$ vs Aβ-treated cells.

However, PSPEa treatment decreased the Aβ_{1-42}-induced ROS production (90.2 ± 10.0%). In addition, PSPEb-treated group also had decreased Aβ_{1-42}-induced ROS production (93.2 ± 9.0%). The results suggested the involvement of ROS in Aβ_{1-42}-induced cytotoxicity and the antioxidative activity of PSPEa and PSPEb in SH-SY5Y cells. However, there was no significant difference between PSPEa and PSPEb treatment groups.

DISCUSSION

Sweet potatoes contain high levels of phenolic compounds that have been reported to have potential for use as a functional food for improving human health, such as inhibition of the growth of human colon, leukemia, and stomach cancer cells.[17] Moreover, PSP has received a lot of attention for its good effects on health[18] because it contains high anthocyanin pigments, CQA derivatives, and various nutrients as compared with other kinds of sweet potato. CQA derivatives have been reported to have cardioprotective effects and exhibit lipid hyperoxidation inhibitory activity and antitumor activity, in addition to its antioxidant effect with free radical scavenging activities.[19,20] Also, from the viewpoint of nutritional science, CQA is known to be poorly absorbed in the intestine but is hydrolyzed by intestinal microflora into various aromatic acid metabolites such as caffeic acid and quinic acid.[21] Caffeic acid has a stronger antioxidative activity than that of CQA, and caffeic acid is readily absorbed in human intestinal epithelium Caco-2 cells as compared to CQA.[22] Moreover, clinical study results also suggest that CQAs are absorbed in the form of caffeic acid conjugates, such as caffeic acid sulfates/glucuronides, in the upper gut. Therefore, in the present study, the caffeic acid and its conjugates in PSP extract may have played an important role in the neuroprotective effect of PSP on SH-SY5Y cells and on the improvement of spatial learning and memory of SAMP8 mice. Analysis of the concentration of caffeic acid and its conjugates in plasma and brain is therefore needed to determine the bioactivity of CQA in the brain that contributes to the improvement of spatial learning and memory. It has been reported that 12.4 ± 1.8 mg/100 g of caffeic acid was detected in the brain of mice with a diet containing 2% caffeic acid for 4 weeks.[23] Moreover, caffeic acid protected the PC12 cells against Aβ-induced cell death through the attenuation of intracellular calcium influx and reduction of τ phosphorylation by the decrease to GSK-3β activation.[24] Thus, caffeic acid in the brain can provide protection against neuronal cell death. It has been reported that PSP also contains anthocyanins that possess numerous biological functions, which include ROS scavenging, antimutagenic, anticarcinogenic, and antihypertensive effects,[25] and improvement of spatial learning and memory.[12,13]

SAMP8 has been used as a model for accelerated senescence in neuroscience research and has provided valuable insights into the physiological and pathological mechanisms underlying neuronal function. Intake of PSPEa and PSPEb for 3 days tended to improve mouse memory (Figure 1A). It was observed that on the seventh day of oral administration of the extract, the SAMP8 escape latency time has increased in the PSPEb-treated group; this implies that the bioactivity of CQA decreases with time, indicating a need for consumption of PSP or CQA to be done continuously to maintain the effective minimum concentration in the system. However, in the PSPEa-administered group, anthocyanins may have an additive effect on the improvement of spatial learning and memory, because

the decrease in the escape latency time was maintained in SAMP8 treated with PSPEa for 7 days. However, SAMP8 treated with PSPEb showed an increase in the escape latency time at the seventh day of the trial (Figure 1A).

According to the free radical theory of aging, the generation of ROS, or free radical-induced oxidative stress, can lead to cell and tissue damage, paralleled by alterations in the function of the genetic apparatus, and results to aging and age-related memory loss.[26,27] In this study, results showed that PSPEa induced the overexpression of PRDX2 protein in the brain of SAMP8 (Figure 2 and Table 3). PRDX2 is one of the antioxidative enzymes that may play a protective role against the oxidative damage caused by ROS.[28] Because PRDX2 modulates the intracellular H_2O_2 production and H_2O_2-mediated apoptosis,[29] it is possible that PRDX2 eliminated the Aβ-mediated H_2O_2 production in neurons. Moreover, PSPEa and PSPEb inhibited the intracellular ROS production (Figure 5) and the neuronal cell death caused by the oxidative stress following Aβ_{1-42} treatment in the human neuroblastoma clonal SH-SY5Y cells (Figure 3). Overexpression of PRDX2 and inhibition of the intracellular ROS production suggests that PSPEa and PSPEb improved the antioxidant system in neurons that resulted in the prevention of biological dysfunction of neurons in SAMP8 mice brain, causing an improvement in learning and memory.

Glucose metabolism is the main source of cerebral energy under normal conditions, and the necessity for glucose in brain function has been established to be solely for ATP production. This line of evidence suggests that glycolysis plays an important role in maintaining normal synaptic function. In the present study, we found that treatment with PSPEa significantly increased the TPI and PGAM1 expressions in the brain of SAMP8 (Figure 2 and Table 3). In AD brain, the protein expressions of PGM1 and TPI were significantly decreased.[30,31] In addition, COX5A was increased in the brain of PSPEa-administered SAMP8 (Table 3). In the electron transport chain, cytochrome oxidase (CcO) converts oxygen to water as the final step, driving aerobic ATP production and therefore supplying most of the phosphorylative energy to the brain. CcO is susceptible mediated to damage by ROS that are generated by cerebral metabolism.[32] In addition, metabolomics analysis revealed that the concentrations of glycolysis- and TCA cycle-related metabolites were increased in PSPEa-treated SH-SY5Y cells (Table 4), and intracellular ATP production was significantly increased in both PSPEa- and PSPEb-treated SH-SY5Y cells (Figure 4). In this study, the overexpression of COX5A, PGAM1, and TPI caused the improvement of glycolytic function and increased ATP production. PSPEa and PSPEb promoted the intracellular ATP production in SH-SY5Y cells, suggesting that PSPEa and PSPEb have a positive effect on neuronal recovery and improved cognitive function. The increase in the rate of energy metabolism presents an alternative pathway for the reduction of the oxidative stress. These results therefore provided an insight on the mechanism of the effect of CQA, and that is the induction of the overexpression of PGK1 gene and increasing the intracellular ATP production.[8]

The exact cause of the observed deficiency in learning and memory during aging is not clear, but some stereological studies in rats have demonstrated that a loss of principal neurons in the hippocampus account for age-related learning and memory impairment.[33] In this study, the protein spots of ACTB, TBB2A, cofilin-1, PPIA, and GFAP were observed to be increased in SAMP8 following treatment with PSPEa (Figure 2 and Table 3). Actin is also a cause involved in the elongation of the growth cone, and a loss of actin function could cause a loss of synapse and neuronal communication in AD.[34] Tubulin is the core protein of microtubules and plays a role in cytoskeletal maintenance. Additionally, tubulin has been shown to be involved in the transport of membrane-bound organelles and is required for extension and maintenance of neurites. A decrease in tubulin may lead to loss of protein function, which could result in the loss of neuronal connections and communication, as well as compromised cellular structure, leading to neurodegeneration.[35] Moreover, cofilin plays an important role in neuronal shape,[36] Peptidyl-prolyl cis/trans isomerase (Pin 1) is the enzyme that catalyzes the isomerization of τ, a neuronal cytoskeleton protein, which is hyperphosphorylated in AD brain.[37] In addition, GFAP is widely used as an astroglial cell marker, and an elevation in GFAP expression indicates enhanced astrogliosis associated with brain aging and dementias.[38] These results suggest that oral administration with PSPEa may enhance the synthesis of cytoskeletal protein and promote neural plasticity by enhancing synapse formation in the brain of SAMP8 mice.

Several polyphenols have been shown to interact with cellular signaling pathways, which are directly or indirectly involved in neuroprotective effects. Generally, most of these pathways are related to antioxidative effects, cell survival, and apoptosis. For example, (−)-epigallocatechin-3-gallate (EGCG) induced overexpression of genes that are important in causing antioxidative effects, such as glutathione *S*-transferase and hemeoxygenase-1 genes.[39,40] Moreover, decreasing the level of antioxidant-related genes may lead to disruption of redox status with subsequent activation of upstream kinases including phosphatidylinositol 3-kinase, protein kinase C, and MAPKs, such as c-Jun NH2-terminal kinase and extracellular signal-regulated kinase.[41] Moreover, another report showed that EGCG induced upregulation of neuronal plasticity-related proteins (β-actin, the actin binding protein tropomyosin 3, and β-tubulin IV).[42] These proteins were known to play crucial roles in developing neurons. From these reports, our proteomics results considered that upregulation of antioxidative effect and neuronal plasticity-related proteins are general mechanisms of the neuroprotective effect. However, overexpression of energy metabolism-related proteins-induced PSPEa was considered the specific mechanism of neuroprotective effect.

In this study, we report that oral administration of PSPEa and PSPEb caused an improvement in the spatial learning and memory on SAMP8 (Figure 1A), and proteomics analysis of the mouse brain revealed that PSPEa increased the expression of proteins relevant for antioxidant effect (PRDX2), energy metabolism (TPI, PGAM1, and COX5A), and neuronal plasticity-related proteins (ACTB, TBB2A, cofilin-1, PPIA, and GFAP) in the brain of SAMP8 (Figure 2C and Table 3). In addition, PSPEa and PSPEb have a neuroprotective effect on Aβ_{1-42}-treated SH-SY5Y cells (Figure 3). PSPEa and PSPEb increased the cell proliferation and the intracellular ATP production (Figure 4), as well as inhibited the intracellular ROS production in Aβ_{1-42}-pretreated SH-SY5Y cells (Figure 5). These results suggest that there are two ways by which PSPEa and PSPEb may prevent neuronal cell death caused by Aβ: (a) promotion of the intracellular ATP production as shown by the PSPEa-increased neuronal cell proliferation and (b) countering the oxidative stress with PSPEa/b's antioxidant effect. These results, therefore, suggest that the effect of PSP extract on the

improvement of spatial learning and memory of SAMP8 mouse may be imparted by its neuroprotective effect. Moreover, comparing PSPEa with PSPEb in terms of their ability to enhance intracellular ATP production, neuroprotective effect, and improve spatial learning and memory, PSPEa was more bioactive, which means that CQAs alone or even in the absence of anthocyanins can improve spatial learning in mice, and when there is anthocyanin (PSPEb), there is an additive effect. A long-term consumption of CQA and anthocyanin-rich PSP is therefore deemed to have a potential in decreasing the incidence of neurodegenerative disorders, as well as in preventing or reversing the deterioration of cognitive performance associated with old age.

AUTHOR INFORMATION

Corresponding Author

*Tel: 81-29-853-5775. Fax: 81-29-853-5776. E-mail: isoda.hiroko.ga@u.tsukuba.ac.jp.

Funding

This study was partially supported by the Japan Science and Technology agency (JST) and the Japan International Cooperation Agency (JICA)'s Science and Technology Research Partnership for Sustainable Development (SATREPS).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr. Abdelfatteh El Omri for giving us wonderful suggestions for the improvement of the manuscript.

ABBREVIATIONS USED

Aβ, amyloid-β; ACTB, actin-β; ATP, adenosine triphosphate; AD, Alzheimer's disease; CcO, cytochrome oxidase; CE-ToFMS, capillary electrophoresis time-of-flight mass spectrometry; COX5A, CcO subunit 5A; CQA, caffeoylquinic acid; DCF, dichlorofluoresein; DCFH-DA, dichlorodihydrofluoresein diacetate; DMEM, Dulbecco's modified Eagle's medium; EGCG, (−)-epigallocatechin-3-gallate; FBS, fetal bovine serum; GFAP, glial fibrillary acidic protein; IPG, immobilized pH gradients; MWM, Morris water maze; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PGAM1, phosphoglycerate mutase 1; Pin 1, peptidyl-prolyl cis/trans isomerase; PPIA, peptidyl-prolyl cis/trans isomerase A; PRDX2, peroxiredoxin-2; PSP, purple sweet potato; PSPEa, PSP extracts; PSPEb, PSP extracts without anthocyanin; ROS, reactive oxygen species; SAMP, senescence-accelerated prone mouse; SAMR, senescence-accelerated resistant mouse; TBB2A, tubulin-β 2A; TCA, tricarboxylic acid; TPI, triosephosphate isomerase

REFERENCES

(1) Mano, H.; Ogasawara, F.; Sato, K.; Higo, H.; Minobe, Y. Isolation of a regulatory gene of anthocyanin biosynthesis in tuberous roots of purple-fleshed sweet potato. *J. Plant Physiol.* **2007**, *143*, 1252−1268.

(2) Furuta, S.; Sada, I.; Nishida, Y.; Yamakawa, O. High *tert*-butylperoxyl radical scavenging activities of sweet potato cultivars with purple flesh. *JSFST* **1998**, *4*, 33−35.

(3) Stintzing, F. C.; Stintzing, A. S.; Carle, R.; Frei, B.; Wrolstad, R. E. Color and antioxidant properties of cyanidin-based anthocyanin pigments. *J. Agric. Food Chem.* **2002**, *50*, 6172−6181.

(4) Huang, G. J.; Sheu, M. J.; Chen, H. J.; Chang, Y. S.; Lin, Y. H. Growth inhibition and induction of apoptosis in NB4 promyelocytic leukemia cells by trypsin inhibitor from sweet potato storage roots. *J. Agric. Food Chem.* **2007**, *55*, 2548−2553.

(5) Tarozzi, A.; Merlicco, A.; Morroni, F.; Franco, F.; Cantelli-Forti, G.; Teti, G.; Falconi, M.; Hrelia, P. Cyanidin 3-O-glucopyranoside protects and rescues SH-SY5Y cells against amyloid-beta peptide-induced toxicity. *NeuroReport* **2008**, *19*, 1483−1486.

(6) Maeda, G. About the characteristics of components and color of the "Okiyumemurasaki" purple sweet potatoes from Okinawa (Japanese). *Res. Rep. OITC* **2006**, *8*, 71−77.

(7) Kim, S. S.; Park, R. Y.; Jeon, H. J.; Kwon, Y. S.; Chun, W. Neuroprotective effects of 3,5-dicaffeoylquinic acid on hydrogen peroxide-induced cell death in SH-SY5Y cells. *Phytother. Res.* **2005**, *19*, 243−245.

(8) Han, J.; Miyamae, Y.; Shigemori, H.; Isoda, H. Neuroprotective effect of 3,5-di-*O*-caffeoylquinic acid on SH-SY5Y cells and senescence-accelerated-prone mice 8 through the up-regulation of phosphoglycerate kinase-1. *Neuroscience* **2010**, *169*, 1039−1045.

(9) Selkoe, D. J. Alzheimer's disease: Genes, protein, and therapy. *Physiol. Rev.* **2001**, *81*, 741−766.

(10) Cross, C. E.; Halliwell, B.; Borish, E. T.; Pryor, W. A.; Ames, B. N.; Saul, R. L.; McCord, J. M.; Harman, D. Oxygen radicals and human disease. *Ann. Intern. Med.* **1987**, *107*, 526−545.

(11) Shimmyo, Y.; Kihara, T.; Akaike, A.; Niidome, T.; Sugimoto, H. Flavonols and flavones as BACE-1 inhibitors: structure-activity relationship in cell-free, cell-based and in silico studies reveal novel pharmacophore features. *Biochim. Biophys. Acta* **2008**, *1780*, 819−825.

(12) Shan, Q.; Lu, J.; Zheng, Y.; Li, J.; Zhou, Z.; Hu, B.; Zhang, Z.; Fan, S.; Mao, Z.; Wang, Y. J.; Ma, D. Purple sweet potato color ameliorates cognition deficits and attenuates oxidative damage and inflammation in aging mouse brain induced by D-galactose. *J. Biomed. Biotechnol.* **2009**, 564737.

(13) Wang, Y. J.; Zheng, Y. L.; Lu, J.; Chen, G. Q.; Wang, X. H.; Feng, J.; Ruan, J.; Sun, X.; Li, C. X.; Sun, Q. J. Purple sweet potato color suppresses lipopolysaccharide-induced acute inflammatory response in mouse brain. *Neurochem. Int.* **2010**, *56*, 424−430.

(14) Sasaki, K.; Han, J.; Isoda, H.; Tamaru, Y.; Wakinosono, M. Neuroprotective effect of extract of purple sweet potato (Japanese). *New Food Industry* **2010**, *52*, 15−20.

(15) Oki, T.; Masuda, M.; Furuta, S.; Nishiba, Y.; Teruhara, N.; Suda, I. Involvement of anthocyanins and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *J. Food Sci.* **2002**, *67*, 1752−1756.

(16) LeBel, C. P.; Ali, S. F.; Makee, M.; Bondy, S. C. Organometal-induced increases in oxygen radical species: The potential of 2,7-dichlorofluorescin diacetate as an index of neurotoxic damage. *Toxicol. Appl. Pharmacol.* **1990**, *104*, 17−24.

(17) Kurata, R.; Adachi, M.; Yamakawa, O.; Yoshimoto, M. Growth suppression of human cancer cells by polyphenolics from sweet potato (*Ipomoea batatas* L.) leaves. *J. Agric. Food Chem.* **2007**, *55*, 185−190.

(18) Lila, M. A. Anthocyanins and human health: An in vitro investigative approach. *J. Biomed. Biotechnol.* **2004**, *5*, 306−313.

(19) Almeida, I. F.; Fernandes, E.; Lima, J. L.; Valentao, P.; Andrade, P. B.; Seabra, R. M.; Coata, P. C.; Bahia, M. F. Oxygen and nitrogen reactive species are effectively scavenged by *Eucalyptus globulus* leaf water extract. *J. Med. Food* **2009**, *12*, 175−183.

(20) Maas, M.; Petereit, F.; Hensel, A. Caffeic acid derivatives from *Eupatorium perfoliatum* L. *Molecules* **2009**, *14*, 36−45.

(21) Gonthier, M. P.; Verny, M. A.; Besson, C.; Remesy, C.; Scalbert, A. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *J. Nutr.* **2003**, *133*, 1853−1859.

(22) Sato, Y.; Itagaki, S.; Kurokawa, T.; Ogura, J.; Kobayashi, M.; Hirano, T.; Sugawara, M.; Iseki, K. In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. *Int. J. Pharm.* **2011**, *403*, 136−138.

(23) Tsai, S. J.; Chao, C. Y.; Yin, M. C. Preventive and therapeutic effects of caffeic acid against inflammatory injury in striatum of MPTP-treated mice. *Eur. J. Pharmacol.* **2011**, *670*, 441−447.

(24) Sul, D.; Kim, H. S.; Lee, D.; Joo, S. S; Hwang, K. W.; Park, S. Y. Protective effect of caffeic acid against beta-amyloid-induced neuro-

toxicity by the inhibition of calcium influx and tau phosphorylation. *Life Sci.* **2009**, *84*, 257−262.
(25) Ahmed, M.; Akter, M. S.; Eun, J. B. Impact of α-amylase and maltodextrin on physicochemical, functional and antioxidant capacity of spray-dried purple sweet potato flour. *J. Sci. Food Agric.* **2010**, *90*, 494−502.
(26) Harman, D. Free-radical theory of aging. *Ann. N. Y. Acad. Sci.* **1994**, *717*, 1−15.
(27) Muller, F. L.; Lustgarten, M. S.; Jang, Y.; Richardson, A.; Van Remmen, H. Trends in oxidative aging theories. *Free Radical Biol. Med.* **2007**, *43*, 477−503.
(28) Ying-Hao, H.; Hyun-Sun, K.; Sang-Keun, K.; Dae-Yeul, Y.; Eun-Yi, M. Inhibitory role of peroxiredoxin II (Prx II) on cellular senescence. *FEBS Lett.* **2005**, *579*, 4897−4902.
(29) Kim, H.; Lee, T. H.; Park, E. S.; Suh, J. M.; Park, S. J.; Chung, H. K.; Kwon, O. Y.; Kim, Y. K.; Ro, H. K.; Shong, M. Role of peroxiredoxins in regulating intracellular hydrogen peroxide and hydrogen peroxide-induced apoptosis in thyroid cells. *J. Biol. Chem.* **2000**, *275*, 18266−18270.
(30) Iwangoff, P.; Armbruster, R.; Enz, A.; Meier-Ruge, W. Glycolytic enzymes from human autoptic brain cortex: normal aged and demented cases. *Mech. Ageing Dev.* **1980**, *14*, 203−209.
(31) Meier-Ruge, W.; Iwangoff, P.; Reichlmeier, K. Neurochemical enzyme changes in Alzheimer's disease and Pick's disease. *Arch. Gerontol. Geriatr.* **1984**, *3*, 161−165.
(32) Paradies, G.; Petrosillo, G.; Pistolese, M.; Ruggiero, F. M. The effect of reactive oxygen species generated from the mitochondrial electron transport chain on the cytochrome c oxidase activity and on the cardiolipin content in bovine heart submitochondrial particles. *FEBS Lett.* **2000**, *466*, 323−326.
(33) Wickelgren, I. Is hippocampal cell death a myth? *Science* **1996**, *271*, 1229.
(34) Masliah, E.; Mallory, M.; Hansen, L.; DeTeresa, R.; Alford, M.; Robert, T. Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci. Lett.* **1994**, *174*, 67−72.
(35) Boyd-Kimball, D.; Sultana, R.; Poon, H. F.; Lynn, B. C.; Casamenti, F.; Pepeu, G.; Klein, J. B.; Butterfield, D. A. Proteomic identification of proteins specifically oxidized by intracerebral injection of amyloid beta-peptide (1−42) into rat brain: implications for Alzheimer's disease. *Neuroscience* **2005**, *132*, 313−324.
(36) Sarmiere, P. D.; Bamburg, J. R. Regulation of the neuronal actin cytoskeleton by ADF/Cofilin. *J. Neurobiol.* **2004**, *58*, 103−117.
(37) Kurt, M. A.; Davies, D. C.; Kidd, M.; Duff, K.; Howlett, D. R. Hyperphosphorylated tau and paired helical filament-like structures in the brains of mice carrying mutant amyloid precursor protein and mutant presenilin-1 transgenes. *Neurobiol. Dis.* **2003**, *14*, 89−97.
(38) Nichols, N. R. Glial responses to steroids as markers of brain aging. *J. Neurobiol.* **1999**, *40*, 585−601.
(39) Chou, F. P.; Chu, Y. C.; Hsu, J. D.; Chiang, H. C.; Wang, C. J. Specific induction of glutathione S-transferase GSTM2 subunit expression by epigallocatechin gallate in rat liver. *Biochem. Pharmacol.* **2000**, *60*, 643−650.
(40) Wu, C. C.; Hsu, M. C.; Hsieh, C. W.; Lin, J. B.; Lai, P. H.; Wung, B. S. Upregulation of hemeoxygenase-1 by Epigallocatechin-3-gallate via the phosphatidylinositol 3-kinase/Akt and ERK pathways. *Life Sci.* **2006**, *78*, 2889−2897.
(41) Na, H. K.; Surh, Y. J. Modulation of Nrf2-mediated antioxidant and detoxifying enzyme induction by the green tea polyphenol EGCG. *Food Chem. Toxicol.* **2008**, *46*, 1271−1278.
(42) Weinreb, O.; Amit, T.; Moussa, B. H.; Youdim, M. B. A novel approach of proteomics and transcriptomics to study the mechanism of action of the antioxidant−iron chelator green tea polyphenol (−)-epigallocatechin-3-gallate. *Free Radical Biol. Med.* **2007**, *43*, 546−556.