DOI:10.1021/jf1009318

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

s-Ethyl Cysteine and *s*-Propyl Cysteine Alleviate β-Amyloid Induced Cytotoxicity in Nerve Growth Factor Differentiated PC12 Cells

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β-Amyloid peptide (Aβ) was used to induce cytotoxicity in nerve growth factor differentiated PC12 cells, and the effects of *s*-ethyl cysteine (SEC) and *s*-propyl cysteine (SPC) on anti-inflammatory protection, DNA fragmentation, mitochondrial membrane potential (MMP), and activity of Na^+-K^+-ATPase and caspases were examined. Aβ treatment significantly decreased cell viability and MMP, and increased lactate dehydrogenase (LDH) activity and DNA fragmentation ($P < 0.05$). The pretreatments from SEC or SPC at 2.5, 5, and 10 μM significantly enhanced cell viability and MMP, and lowered LDH activity and DNA fragmentation ($P < 0.05$). Aβ treatment also significantly decreased Na^+-K^+-ATPase activity and enhanced the activity of caspase-3 and caspase-8 ($P < 0.05$); however, the pretreatments from SEC or SPC significantly attenuated Aβ-induced reduction in Na^+-K^+-ATPase activity and elevation in caspase-3 and caspase-8 activities ($P < 0.05$). Aβ treatment increased the protein production and mRNA expression of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α ($P < 0.05$). The pretreatments from SEC at 10 μM or SPC at 2.5, 5, and 10 μM significantly suppressed mRNA expression and decreased the protein production of these cytokines. These results suggested that SEC and SPC were potent neuroprotective agents against Alzheimer's disease.

KEYWORDS: Aβ; *s*-ethyl cysteine; *s*-propyl cysteine; Na^+-K^+-ATPase; caspase activity; cytokine

INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the most common form of dementia in many countries. The amyloid cascade hypothesis considers that the β-amyloid peptide (Aβ) plays a central role in the pathogenesis of AD (*1*, *2*). It has been documented that Aβ deposition caused free radical generation and initialized inflammatory damage, which consequently led to neuronal cell apoptosis (*3*−*5*). Vukic et al. (*5*) reported that Aβ could evoke gene expression of several inflammatory cytokines such as monocyte chemoattractant protein-1, interleukin (IL)-1β, and IL-6 in cultured human brain endothelial cells. Yamamoto et al. (*6*) indicated that glial interferon-γ and tumor necrosis factor (TNF)-α increased Aβ deposition through enhancing BACE1 expression and suppressing Aβ clearance. Apparently, Aβ and inflammatory cytokines are closely interrelated and both contribute to the progression of AD. Therefore, any agents with anti-inflammatory activities might potentially protect neurons against Aβ-induced neurotoxicity and enhance neuronal cell survival.

s-Ethyl cysteine (SEC) and *s*-propyl cysteine (SPC) are hydrophilic cysteine-containing compounds naturally formed in *Allium* plants such as garlic and onion (*7*, *8*). Our previous study has reported that the preintake of SEC and SPC markedly protected mice brains against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson-like oxidative and inflammatory injury via increasing retention of dopamine and glutathione, elevated GPX mRNA expression, and diminished TNF-α mRNA expression (*9*). Another study of ours further observed that these compounds protected PC12 cells against H_2O_2-caused oxidative and apoptotic damage via the retention of mitochondrial membrane potential (MMP) and decreasing DNA fragmentation (*10*). These findings support that both SEC and SPC, via acting as antioxidative and anti-inflammatory agents, are able to prevent or alleviate cytotoxicity and oxidative stress occurring in neurodegenerative disorders. However, it is unknown as to whether these compounds benefit neuronal cells against Aβ-induced apoptosis and inflammatory stress. If these compounds could attenuate Aβ-induced neurotoxicity, then they may be developed as anti-Alzhemer's agents.

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 Published on Web 05/05/2010

The PC12 cell line is a rat adrenal gland pheochromocytoma cell line and could differentiate to a sympathetic neuronal phenotype via incubation with nerve growth factor (NGF) (*11*). This NGF differentiated PC12 cell line has been widely used as a sympathetic neuron model for studying the survival of neuronal cells or antioxidant protection (*12*). In our present study, Aβ was used to induce cytotoxicity in NGF differentiated PC12 cells, and the concentration effects of SEC and SPC on cell viability, MMP, DNA fragmentation, and the activity of Na^+-K^+-ATPase and caspases were investigated. The influence of SEC and SPC upon protein production and mRNA expression of inflammatory cytokines was also examined.

MATERIALS AND METHODS

Chemicals. SEC (99.5%) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). SPC (99%) was supplied by Wakunaga Pharmaceutical Co. (Hiroshima, Japan). Medium, plates, antibiotics, and chemicals used for cell culture were purchased from Difco Laboratory (Detroit, MI, USA). All chemicals used in measurements were of the highest purity commercially available. Nerve growth factor (NGF) was purchased from Promega Co. (Madison, WI, USA). β-Amyloid peptide ($A\beta_{1-42}$) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). This peptide was diluted in 10 mM sodium phosphate buffer (PBS, pH 7.2) to a final concentration for experiments.

Cell Culture. PC12 cells were cultured in 35 mm dish containing Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated calf serum, 5% fetal bovine serum, 100 units/mL of penicillin, and 100 units/mL of streptomycin under 95% air and 5% CO_2 at 37 °C. PC12 cells were treated with NGF (50 ng/mL) and allowed to differentiate for 5 days. The culture medium was changed every three days, and cells were subcultured once a week. The medium was changed to serum-deprived medium, and cells were washed with serum-free DMEM 24 h before experiments and replanted in the 96 well plates.

Experimental Design. SEC or SPC was prepared with the medium. NGF differentiated PC12 cells (10^5 cells/mL) were pretreated with SEC or SPC at 1.25, 2.5, 5, or 10 μM for 48 h at 37 °C, followed by 24 h of exposure to 10 μM Aβ at 37 °C.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide (MTT) Assay. MTT assay was performed to examine cell viability. MTT (99.5%) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Briefly, control or treated PC12 cells were incubated with 0.25 mg of MTT/mL for 3 h at 37 °C. The amount of MTT formazan product was determined by measuring absorbance at 570 nm (630 nm as a reference) using a microplate reader (Bio-Rad, Hercules, CA, USA). Cell viability was expressed as a percentage of control without the addition of SEC or SPC and without Aβ treatment.

Lactate Dehydrogenase (LDH) Assay. The plasma membrane damage of PC12 cells was evaluated by measuring the activity of intracellular LDH in the medium. Fifty microliters of culture supernatant was collected from each well, and LDH activity (U/L) was determined by a colorimetric LDH assay kit (Sigma Chemical Co., St. Louis, MO, USA). The intra-assay coefficient of variation (CV) was 6.3%, and the interassay CV was 5.9%.

Measurement of DNA Fragmentation. Cell death detection ELISA plus kit (Roche Molecular Biochemicals, Mannheim, Germany) was used to quantify DNA fragmentation. The intra-assay CV was 4.6%, and the interassay CV was 5.1%. PC12 cells were lysed for 30 min at room temperature, followed by centrifugation at 200*g* for 10 min. Then, 20 μL of supernatant was transferred onto the streptavidin-coated plate, and 80 μL of freshly prepared immunoreagent was added to each well and incubated for 2 h at room temperature. After washing twice with PBS (pH 7.2), we added the substrate solution and incubated the wells for 15 min. The absorbance at 405 nm (reference wavelength 490 nm) was measured using a microplate reader. DNA fragmentation was expressed as the enrichment factor using the following equation:

$$\text{enrichment factor} = (\text{absorbance of the sample})/(\text{absorbance of the control})$$

where sample = cells treated with Aβ or/and test compound; control = cells without Aβ and test compound treatment.

Determination of Mitochondrial Membrane Potential (MMP). MMP was monitored using flow cytometry (Beckman-FC500, Beckman Coulter, Fullerton, CA, USA) and the fluorescent dye Rhodamine123 (Rh123) purchased from Sigma Chemical Co. (St. Louis, MO, USA) on the basis of the depolarization of MMP resulting in the loss of Rh123 from the mitochondria and causing a decrease in intracellular fluorescence. Rh123 (100 μg/L) was added to PC12 cells for 45 min at 37 °C. Then, cells were collected and washed twice with PBS (pH 7.2). The mean fluorescence intensity (MFI) in PC12 cells were analyzed with flow cytometry.

Na^+-K^+-ATPase Activity Assay. Na^+-K^+-ATPase activity was determined by measuring the amount of inorganic phosphate (Pi) released from ATP (*13*). The reaction mixture contained 100 mM NaCl, 20 mM KCl, 2 mM ATP, 30 mM Tris-HCl buffer (pH 7.4), and the fresh cellular mitochondria isolated according to the method of Ashraf et al. (*14*). This assay was initiated by adding ATP and terminated by adding 15% trichloroacetic acid after 15 min of incubation at 37 °C. The released inorganic phosphate was assayed by measuring the absorbance at 640 nm. A unit was defined as 1 μmol of Pi released from ATP by 1 mg of protein during 1 h. The values of the treated cells were normalized against the value of the control and expressed as a percentage of control.

Measurement of Caspases Activity. Activity of caspase-3 and -8 was detected by using fluorometric assay kits (Upstate, Lake Placid, NY, USA) according to the manufacturer's protocol. The intra-assay CV was 3.4−4.9%, and the interassay CV was 5.2−7.0%. Briefly, control or treated cells were lysed in 50 mL of cold lysis buffer and incubated in ice for 10 min. Fifty microliters of cell lysates was mixed with 50 mL of reaction buffer and 5 mL of fluorogenic substrates specific for caspase-3 or -8 in a 96-well microplate. After incubation at 37 °C for 1 h, we measured fluorescent activity using a fluorophotometer with excitation at 400 nm and emission at 505 nm. Data were expressed as a percentage of the control.

IL-1β, IL-6, and TNF-α Analyses. After Aβ treatment, we washed the cells twice with PBS and homogenized them with 2 mL of PBS (pH 7.2) in a motor driven Teflon glass homogenizer (Glas-Col Co., CA, USA), and the released levels of IL-1β, IL-6, and TNF-α in supernatant were measured by ELISA methods using cytoscreen immunoassay kits (BioSource Intl., Camarillo, CA, USA). The sensitivity of the assay with the detection limit was 5 pg/mL for IL-1β, IL-6, and 10 pg/mL for TNF-α.

Real-Time Polymerase Chain Reaction (RT-PCR) for mRNA Expression. RT-PCR was used to examine the mRNA expression of IL-1β, IL-6, and TNF-α. PC 12 cells were homogenized in guanidinethiocyanate, and RNA was extracted using TRIizol reagent and further digested with DNase, and quantified by measuring the absorbance at 260 nm. Two micrograms of total RNA was used to generate cDNA. Reverse transcription was performed in a one-step protocol using the iScript cDNA Synthesis Kit (Bio-Rad Co., Hercules, CA, USA) according to the manufacturer's instructions. The primers were as follows (*15*, *16*). TNF-α: forward, 5′-GAT CTC AAA GAC AAC CCA ACT AGT-3′; reverse, 5′-CTC CAG CTG GAA GAC TCC TCC CAG-3′. IL-6: forward, 5′-CAA GAG ACT TCC AGC CAG TTG C-3′; reverse, 5′-TTG CCG AGG TAG ACC TCA TAG TGA CC-3′. IL-1β: forward, 5′-ATG GCA ACT GTT CCT GAA CTC AAC T-3′; reverse, 5′-AGG ACA GGT ATA GAT TCT TTC CTT T-3′. mRNA levels were standardized by using the primers of glyceraldehyde-3-phosphate dehydrogenase (GAPDH): forward, 5′-AAT GTG TCC GTC GTG GAT CTG A-3′; reverse, 5′-GAT GCC TGC TTC ACC ACC TTC T-3′. Real-time PCR reactions were performed using the ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The following temperature parameters were cycled 40 times: 15 s at 95 °C and 1 min at 60 °C. In this present study, the mRNA level of the control group (without Aβ and test compound) was defined as 100%; then, the mRNA level of other groups was calculated as a percentage of the control group.

Statistical Analysis. The effect of each treatment was analyzed from nine different preparations (n = 9). Data were reported as means ± standard deviation (SD) 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

Four concentrations of SEC or SPC were used in this study. However, neither SEC nor SPC at 1.25 μM showed any effect in

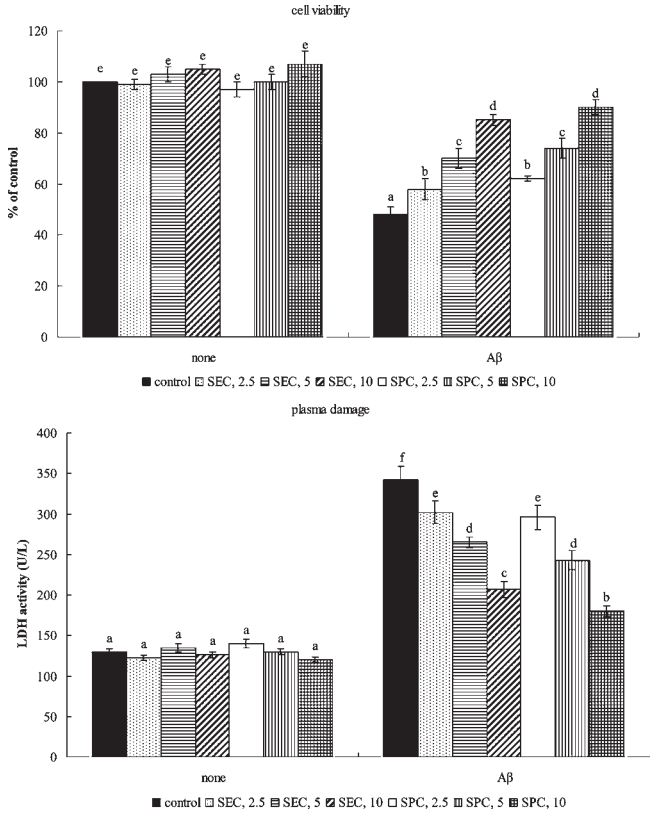

Figure 1. Effect of SEC or SPC upon cell viability and plasma membrane damage, determined by MTT assay and LDH assay. NGF differentiated PC12 cells were pretreated with SEC or SPC at 2.5, 5, or 10 μM followed by the addition of 10 μM Aβ to induce cell death. The control contained no SEC or SPC. None means that Aβ was not added. Data are mean $\pm$ SD (n = 9). (a−f) indicate that bars without a common letter differ; $P < 0.05$.

Table 1. Effect of SEC or SPC upon DNA Fragmentation, Determined as Enrichment Factor, and MMP, Determined as MFI[a]

	enrichment factor	MFI
control[b]	1.00 a	100 d
SEC, 10	1.03 $\pm$ 0.06 a	99 $\pm$ 3 d
SPC, 10	0.97 $\pm$ 0.04 a	98 $\pm$ 5 d
Aβ	2.53 $\pm$ 0.21 e	24 $\pm$ 6 a
SEC, 2.5 + Aβ	2.18 $\pm$ 0.13 d	39 $\pm$ 4 b
SEC, 5 + Aβ	2.05 $\pm$ 0.18 d	47 $\pm$ 5 b
SEC, 10 + Aβ	1.67 $\pm$ 0.15 c	70 $\pm$ 8 c
SPC, 2.5 + Aβ	2.13 $\pm$ 0.16 d	38 $\pm$ 3 b
SPC, 5 + Aβ	1.92 $\pm$ 0.20 d	45 $\pm$ 3 b
SPC, 10 + Aβ	1.34 $\pm$ 0.14 b	68 $\pm$ 7 c

[a] NGF differentiated PC12 cells were pretreated with SEC or SPC at 2.5, 5, or 10 μM followed by the addition of 10 μM Aβ to induce cell damage. Data are mean $\pm$ SD (n = 9). Least-square means with a common letter within a column are not different at the 5% level. [b] Controls contained no SEC or SPC.

all measurements (data not shown, $P > 0.05$). The effects of SEC or SPC upon cell viability (determined by MTT assay) and plasma membrane integration (determined by LDH assay) are presented in **Figure 1**. Without Aβ treatment, the addition of SEC or SPC did not affect cell viability or membrane integration ($P > 0.05$). Compared with the control group, Aβ treatment significantly decreased cell viability and increased LDH release ($P < 0.05$). However, the pretreatments from SEC or SPC significantly and concentration-dependently mitigated subsequent Aβ-induced cell death and membrane damage ($P < 0.05$).

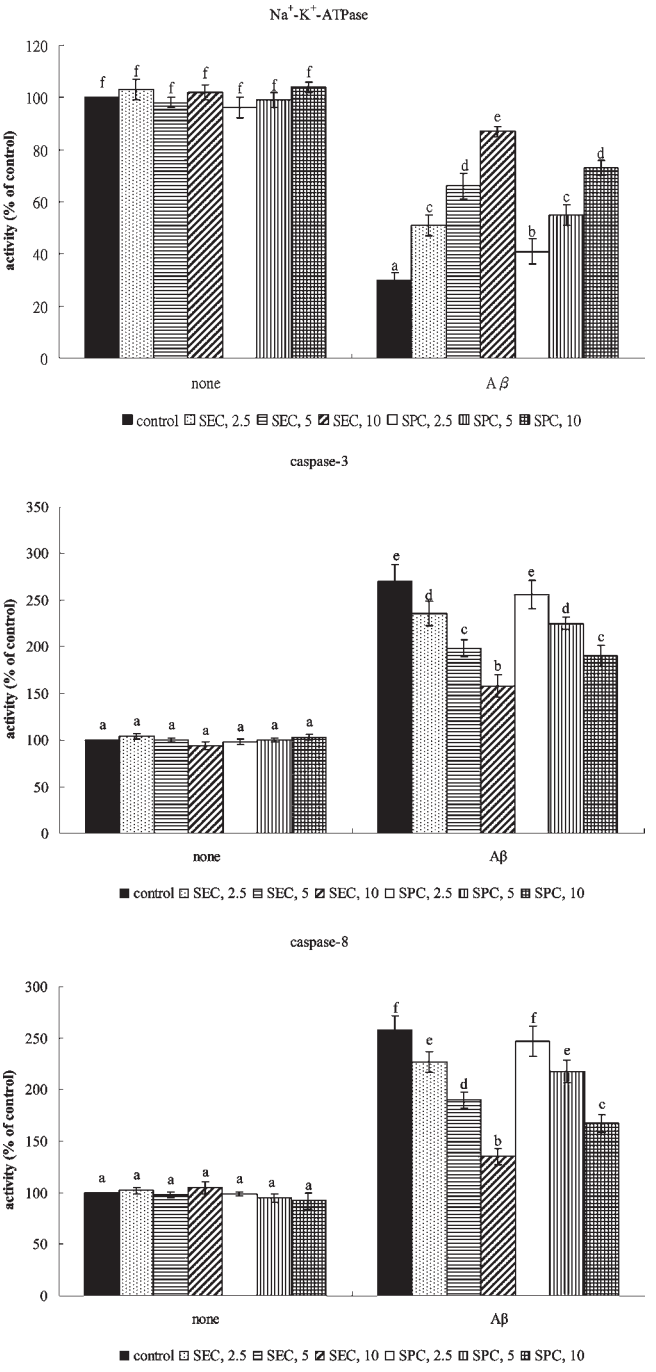

Figure 2. Effect of SEC or SPC upon the activity of Na^+-K^+-ATPase, caspase-3, and caspase-8. NGF differentiated PC12 cells were pretreated with SEC or SPC at 2.5, 5, or 10 μM followed by the addition of 10 μM Aβ to induce cell injury. The control contained no SEC or SPC. None means that Aβ was not added. Data are mean $\pm$ SD (n = 9). (a−f) indicate that bars without a common letter differ; $P < 0.05$.

The effects of SEC or SPC upon DNA fragmentation, determined as the enrichment factor, and MMP, determined as the MFI, are shown in **Table 1**. Compared with the control group, Aβ treatment significantly increased DNA fragmentation and decreased MMP ($P < 0.05$). Compared with Aβ treatment alone, the pretreatments from SEC or SPC significantly decreased Aβ-induced DNA fragmentation and maintained MMP in NGF differentiated PC12 cells ($P < 0.05$). The effects of SEC or SPC upon the activity of Na^+-K^+-ATPase, caspase-3, and caspase-8 are presented in **Figure 2**. Without Aβ treatment, the addition of these

Table 2. Effect of SEC or SPC upon the Protein Level (pg/mL) of IL-1β, IL-6, and TNF-α[a]

	IL-1β	IL-6	TNF-α
control[b]	28 ± 6 a	24 ± 5 a	19 ± 3 a
SEC, 10	23 ± 5 a	26 ± 2 a	21 ± 5 a
SPC, 10	20 ± 7 a	23 ± 4 a	18 ± 6 a
Aβ	207 ± 23 e	246 ± 21 e	257 ± 26 e
SEC, 2.5 + Aβ	196 ± 14 e	237 ± 15 e	244 ± 20 e
SEC, 5 + Aβ	176 ± 17 d	215 ± 20 d	210 ± 21 d
SEC, 10 + Aβ	130 ± 13 c	174 ± 19 c	172 ± 17 c
SPC, 2.5 + Aβ	163 ± 18 d	208 ± 15 d	200 ± 13 d
SPC, 5 + Aβ	140 ± 15 c	180 ± 12 c	175 ± 18 c
SPC, 10 + Aβ	85 ± 10 b	143 ± 8 b	138 ± 14 b

[a] NGF differentiated PC12 cells were pretreated with SEC or SPC at 2.5, 5, or 10 μM and followed by the addition of 10 μM Aβ to induce cell damage. Data are mean ± SD (n = 9). Least-square means with a common letter within a column are not different at the 5% level. [b] Controls contained no SEC or SPC.

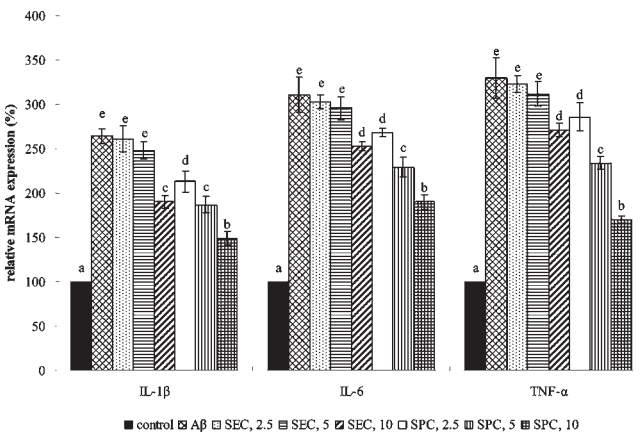

Figure 3. Effect of SEC or SPC upon mRNA expression of IL-1β, IL-6, and TNF-α. NGF differentiated PC12 cells were pretreated with SEC or SPC at 2.5, 5, or 10 μM followed by the addition of 10 μM Aβ to induce cell injury. The control contained no SEC or SPC. Data are mean ± SD (n = 9). (a−e) indicate that bars without a common letter differ; $P < 0.05$.

agents did not affect the activity of Na^+-K^+-ATPase, caspase-3, and caspase-8 ($P > 0.05$). Compared with the control group, Aβ treatment significantly decreased Na^+-K^+-ATPase activity and enhanced caspase-3 and caspase-8 activities ($P < 0.05$). However, the pretreatments from these agents significantly restored Na^+-K^+-ATPase activity and diminished caspase-3 and caspase-8 activities ($P < 0.05$), in which SEC at 10 μM showed the greatest effect in retaining Na^+-K^+-ATPase activity and reducing caspase-3 and caspase-8 activities ($P < 0.05$).

As shown in **Table 2** and **Figure 3**, without Aβ treatment, the addition of SEC or SPC did not affect the release of IL-1β, IL-6, and TNF-α in NGF differentiated PC12 cells ($P > 0.05$). Compared with the control group, Aβ treatment significantly increased the protein production and mRNA expression of three test inflammatory cytokines ($P < 0.05$). SEC pretreatments at 5 and 10 μM significantly lowered IL-1β, IL-6, and TNF-α production but only at 10 μM significantly suppressed Aβ-induced mRNA expression of three cytokines ($P < 0.05$). The pretreatments from SPC significantly and concentration-dependently decreased subsequent Aβ-induced release of IL-1β, IL-6, and TNF-α, as well as suppressed Aβ-induced mRNA expression of test cytokines ($P < 0.05$).

DISCUSSION

It has been documented that Aβ deposition and accumulation could initiate the pathogenesis of Alzheimer's disease via triggering a cascade of events such as τ-phosphorylation and inflammatory injury (*17*, *18*). The results of our present study revealed that the pretreatments from SEC or SPC at 2.5, 5, and 10 μM effectively protected NGF differentiated PC12 cells against Aβ-induced cell death, membrane damage, DNA fragmentation, mitochondrial dysfunction, and inflammatory cytokine production. These findings suggested that SEC and SPC could penetrate NGF differentiated PC12 cells and exert their protective actions against Aβ-induced neurotoxicity.

Na^+-K^+-ATPase is a heterodimer composed of an α-subunit and a β-subunit, and the α-subunit is a transmembrane protein responsible for exchanging intracellular Na^+ and extracellular K^+. The decreased Na^+-K^+-ATPase activity implied the collapse of the mitochondrial membrane, which might consequently cause apoptotic insult, cell damage, and/or cell death (*19*). Our present study found that Aβ treatment markedly reduced Na^+-K^+-ATPase activity in NGF treated PC12 cells, which indicated that mitochondrial membranes of these cells lost their function for Na^+/K^+ exchange. Thus, the observed mitochondrial membrane potential reduction in Aβ-treated PC12 cells could be explained. However, it is reported that activation of caspase-3 and caspase-8 could act as an apoptotic executor of cell death in PC12 cells (*20*, *21*) because these caspases are directly responsible for the change in cell morphological events and for the cleavage of nuclear protein poly(ADP-ribose) polymerase (PARP) (*22*). In our present study, the presence of Aβ enhanced both caspase-3 and caspase-8 activities, which might lead to PARP cleavage in PC12 cells. Thus, the Aβ-induced increase in DNA fragmentation and decrease in cell viability that we observed could be partially explained. However, we found that the pretreatments from SEC and SPC effectively alleviated the decline of Na^+-K^+-ATPase activity as well as the elevation of caspae-3 and caspase-8 activities caused by Aβ, which could subsequently stabilize transmembrane protein and mitochondria, benefit Na^+/K^+ exchange, maintain mitochondrial membrane potential, and diminish DNA fragmentation. Therefore, the observed greater cell survival presented in SEC- or SPC-treated PC12 cells could be partially ascribed to these compounds that protect the mitochondrial membrane and maintain Na^+/K^+ exchange.

Enhanced inflammatory stress is one of the major hallmarks of Alzheimer's disease, and the inflammatory process is usually considered to be a downstream effect of the accumulated proteins such as Aβ and τ (*18*, *23*). The clinical significance of IL-1β, IL-6, and TNF-α in plasma or cerebrospinal fluid from AD patients has been observed (*24*−*26*). Jana et al. (*27*) reported that Aβ elicited the gene expression of IL-1β and IL-6 in microglial cells. Our present study further indicated that Aβ treatment markedly evoked mRNA expression of IL-1β, IL-6, and TNF-α, and led to a significant production of these cytokines in NGF differentiated PC12 cells. Obviously, these cytokines were involved in Aβ-initiated neurotoxicity. Furthermore, we found that pretreatments from SEC at 10 μM or SPC at 2.5, 5, and 10 μM effectively counteracted Aβ-induced inflammatory reactions via suppressing mRNA expression and lowering the protein production of these cytokines. These findings suggest that SEC or SPC could protect neuronal cells via inhibiting inflammatory injury, as well as reduce the downstream effect of Aβ in the pathological development via slowing down inflammatory process. Since SEC and SPC could mediate the mRNA expression of three test cytokines, these agents might exert their functions at the level of transcription.

Our previous study has indicated that SEC and SPC could provide antioxidative protection toward PC12 cells via decreasing reactive oxygen species formation and retaining glutathione level (*10*). Thus, it is highly possible that these compounds also offered antioxidative protection to these NGF differentiated

PC12 cells against Aβ-induced cytotoxicity. In addition, it is interesting to find that the protective effects from these agents in NGF treated PC12 cells was different because SEC was more effective in restoring Na^+-K^+-ATPase activity and decreasing caspases activity, but SPC was marked in suppressing mRNA expression and protein production of three cytokines. It seems that the ethyl group of SEC and propyl group of SPC played important roles in determining their bioactivities, and it is possible that this ethyl or propyl group affects the affinity of SEC or SPC toward certain enzymes, carriers, receptors, or channels. These two compounds are hydrophilic peptide derivates and naturally form in *Allium* foods such as garlic. Further studies are necessary to ensure the safety of these agents before they are applied to humans. It is known that the content of these cysteine-containing compounds in *Allium* plants is dependent on the species or vegetation period (*28*). Thus, it may not be always possible to obtain these compounds by supplementing the diet with garlic or other *Allium* plants.

In conclusion, the pretreatments from *s*-ethyl cysteine or *s*-propyl cysteine effectively alleviated inflammatory stress and apoptosis in NGF differentiated PC12 cells against Aβ-induced injury, which finally enhanced cell survival. These agents exhibited anti-inflammatory and antiapoptotic activities via enhancing Na^+-K^+-ATPase activity, diminishing caspase-3 and caspase-8 activities, and suppressing mRNA expression of inflammatory cytokines. These findings support the fact that these compounds are potent anti-Alzheimer's disease agents.

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Received for review March 10, 2010. Revised manuscript received April 26, 2010. Accepted April 27, 2010.