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ScienceDirect
Neuroscience Research 56 (2006) 204–212
Neuroscience Research
www.elsevier.com/locate/neures

Inhibitory effect of sesaminol glucosides on lipopolysaccharide-induced NF-κB activation and target gene expression in cultured rat astrocytes

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Received 14 August 2005; accepted 12 April 2006
Available online 13 July 2006

Abstract

The inflammatory reaction plays an important role in the pathogenesis of the neurodegenerative disorder including Alzheimer's disease (AD). Sesame lignan compounds such as sesaminol glucosides (SG) exhibit a range of pharmacological activities including anti-oxidative and anti-inflammatory action. In this study, we tried to elucidate possible effects of SG on lipopolysaccharide (LPS)-induced inflammatory reaction and its underlying mechanism in cultured astrocytes. SG (10–100 μg/ml) inhibited LPS-induced generation of nitric oxide (NO) and reactive oxygen species (ROS), as well as inhibited LPS-induced cytosolic phospholipase A_2 ($cPLA_2$), cyclooxygenase 2 (COX-2) and inducible nitric oxide synthase (iNOS) expression dose-dependently. This inhibitory effect of SG on NO and ROS generation was enforced by addition of glutathione (GSH) in culture. In addition, SG prevented LPS-induced DNA binding and transcriptional activity of nuclear factor KappaB (NF)-κB. Consistent with the inhibitory effect on NF-κB activity, SG inhibits phosphorylation and degradation of inhibitory KappaB (IκB), thereby translocation of p50 of NF-κB. These data show that SG has an anti-inflammatory effect through inhibition of NF-κB, and may be a useful agent for prevention of inflammatory disease like AD.

Keywords: Sesaminol glucosides; Lipopolysaccharide; NF-κB; Inflammatory reaction; Astrocytes; Alzheimer's disease

1. Introduction

Alzheimer's disease (AD) is the most prevalent form of neurodegenerative disease characterized by deterioration of recognition ability and memory function (Ashe, 2001; Mattson, 2004). Classic pathologic feature of AD includes formation of amyloid plaques and neurofibillary tangles, and neuronal cell death by inflammation (Irizarry and Hyman, 2001). This feature supports the involvement of inflammation in the development of AD (Irizarry and Hyman, 2001; Tuppo and Arias, 2005). Such inflammatory responses are also observed in several other chronic and acute neurodegenerative diseases including Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, and stroke (Torreilles et al., 1999; Matsuoka et al., 2001; Allan and Rothwell, 2003; Cacquevel et al., 2004). The major players of in the inflammatory process in the development of AD are thought to be the astrocytes and possibly to a less extent the neurons, all of which are cellular components of the brain. Astrocytes not only supply nutrients to neurons but also mediate inflammatory responses in the CNS. They respond to pro-inflammation with transcriptional activation of genes for $cPLA_2$, COX-2 and iNOS (Hewett, 1999; Li et al., 1999; Xu et al., 2002).

Increase of the inflammatory genes such as COX-2 and iNOS expression is induced by progressive neural damage after excitotoxicity, ischemia insults, and in the traumatic injury, ischemia, multiple sclerosis, Parkinson's disease and AD (Acarin et al., 2002; Rose et al., 2004). AD, a developmental or progressive neuro-inflammatory disease, is related to the

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0168-0102/$ – see front matter
doi:10.1016/j.neures.2006.06.005

activation of microglia and astrocytes, which induce pro-inflammatory molecules (McGeer and McGeer, 2003; Tuppo and Arias, 2005). Astrocytes can be activated by LPS, cytokines and scopolamine which are commonly used as inducers of AD-like cognitive dysfunction in animal models (Jain et al., 2002; McGeer and McGeer, 2003; Tuppo and Arias, 2005). This has led to the hypothesis that brain inflammation is a cause of neuronal injury in AD and that anti-inflammatory drugs may act as protective agents.

The activation of transcriptional factor NF-κB that regulates expression of COX-2 and iNOS is associated with neuroinflammatory responses (Yamamoto and Gaynor, 2001). NF-κB exists in the cytosol as an inducible three-subunit complex consisting of the transcription factor dimer (p50 and p65) and an associated inhibitory subunit called IκB (Castro-Alcaraz et al., 2002). NF-κB activation occurs when IκB is induced to dissociate from the complex, a process that may involve phosphorylation, proteolysis, and/or oxidative damage to IκB (Magnani et al., 2000; Castro-Alcaraz et al., 2002). Several lines of evidence have demonstrated that agents inhibiting NF-κB can prevent cell death induced by inflammatory insults including exposure to LPS, whereas, IκB decoy DNA (which blocks NF-κB activity) enhances cell death in several neurodegenerative disease like AD (Magnani et al., 2000; Castro-Alcaraz et al., 2002). NF-κB activity was altered in vulnerable brain regions in AD patients (Boissiere et al., 1997). Increasing evidences have demonstrated that inhibition of NF-κB could be benefit for the treatment of AD (Yamamoto and Gaynor, 2001; Song et al., 2004). Astrocytes can readily respond to pro-inflammatory agents, and LPS causes the induction of a number of genes through activation of the NF-κB (Pawate et al., 2004). Therefore, agents inhibiting NF-κB can be useful for the treatment of AD.

The sesame seed (*Sesamum indicum* Linn, Pedaliaceae), has long been used extensively as a traditional health food in East Asia and reveals numerous biological activity (Kang et al., 1999; Ohtsuki et al., 2003). Sesame seed contains large quantities of lignan glucosides (Ohtsuki et al., 2003), such as pinoresinol glucosides (Kang et al., 1999) and sesaminol glucosides (Katsuzaki et al., 1994). The lignans have strong pharmacological activities such as anti-oxidative and anti-inflammatory properties (Katsuzaki et al., 1994; Kang et al., 1999; Hou et al., 2003). Sesaminol shows inhibitory effect on endogenous lipid peroxidation as well as oxidative DNA damage in rat plasma and liver (Ikeda et al., 2003). Sesaminol also has inhibitory effect on inflammatory hepatic ischemia-reperfusion injury in rat (Utsunomiya et al., 2003). We recently found that sesaminol glucosides (SG) has protective effects against $A\beta_{25-35}$-induced learning and memory deficit in mice (Kim et al., 2003), and Aβ-induced cell death in cultured PC12 cells through anti-oxidative mechanism (Lee et al., 2005a). However, anti-inflammatory effect of this compound on neurodegenerative diseases has not been yet elucidated in detail. This study was aimed to determine whether SG has inhibitory effect on LPS-induced inflammatory reaction in cultured rat astrocytes.

2. Materials and methods

2.1. Materials

SG was prepared from refined sesame and purified as described elsewhere (Katsuzaki et al., 1994). Briefly, we prepared SG after stripping off all the oils by extraction with *n*-hexane. Crude SG were extracted with distilled water at 95 °C from defatted sesame flour, and further purified by Diaion HP20 column using 60% ethanol. LPS was purchased from Sigma Chemicals (St. Louis, MO, USA). LPS was dissolved in deionized water at a final concentration of 1 mg/ml and stored at −20 °C. The NO Assay System was purchased from Sigma Chemicals. The Luciferase and Electrophoretic Mobility Shift Assay System were purchased from Promega, Inc. (Chatsworth, CA, USA). The $cPLA_2$, p50, IκB and pIκB antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). The COX-2, iNOS and β-actin antibodies were purchased from Cayman Chemical (Ann Arbor, MI, USA).

2.2. Primary cell culture

Astrocytes isolated from neonatal rat brain (day 1) were incubated for 15 min in Ca^{2+}- and Mg^{2+}-free Hanks' balanced saline solution (Life Technologies, Inc.) containing 0.2% trypsin (Gibco BRL, Grand Island, NY, USA). The cells were dissociated by trituration and plated into polyethyleneimine-coated plastic or glass-bottom culture dishes (5×10^6 cells/100 mm dish) containing minimum essential medium with Earle's salts supplemented with 5% heat-inactivated fetal bovine serum (Gibco BRL, Grand Island, NY, USA), 2 mM L-glutamine, 1 mM pyruvate, 20 mM KCl, 10 mM sodium bicarbonate, and 1 mM Hepes (pH 7.2). Following cell attachment (3–6 h after plating), the culture medium was replaced with DMEM containing 5% fetal bovine serum, every 3 days of culture. The cells were cultured for designated time. The cultured cells contain <10% neuronal cells. In this study, 8–9-day-old cells were used.

2.3. Cell viability

The cells were plated in 96-well plates, and cell viability was determined by the conventional 4-(3-(4-lodophenyl)-2-(4-nitrophenyl)-2H-5-terazolio)-1,3-benzene disulfonate (WST-1) reduction assay (Dojin Laboratory, Kumanoto, Japan) as described elsewhere (Park et al., 2005). The cells were exposed with LPS at 1 μg/ml and with/without various concentrations of SG (10–100 μg/ml) for 24 h. The cells were treated with the WST-1 solution (final concentration, 1 mg/ml) for 2 h. The absorbance was measured with a microplate reader (Tecan, sunrise, Salzburg, Austria) at 450 nm. Results were expressed as the percentage of WST-1 reduction.

2.4. Apoptosis detection (DAPI staining)

Apoptotic cells were determined by the morphological changes after 4,6-diamino-2-phenylindole (DAPI) staining under fluorescence microscopic observation (DAS Microscope LEICA DAR, 100× or 200×) as described previously (Lee et al., 2005b). Astrocytes were plated in 8-well plates, and the cells were exposed with LPS (1 μg/ml) with/without different concentration sesaminol glucosides (10–100 μg/ml) for 24 h. Medium was removed, and the cells were washed with PBS followed by addition of 75 mM KCl. After being fixed twice with acetic acid and methanol (1:3) at room temperature for 3 min, dishes were washed twice with PBS, and then incubated at room temperature with DAPI for 20 min. After being washed once with PBS, dishes were coverslipped using Vectashield medium (Vector, Biosys, Compiegne, France). For each determination, three separate 100 cell counts were scored. Apoptosis was expressed as a percentage calculated from the number of cells with apoptotic nuclear morphology divide by the total number of cells.

2.5. Detection of intracellular reactive oxygen species

To monitor intracellular accumulation of ROS, the fluorescent probe DCF-DA and protocol of experimental process described in elsewhere were used (Lee et al., 2005b). Following treatment with LPS (1 μg/ml) for 4 h in

the presence or absence of sesaminol glucosides (10–100 μg/ml), the cells were washed in modified Kreb's buffer containing 145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 4 mM $NaHCO_3$, 5.5 mM glucose, 10 mM Hepes, pH 7.4. The cell suspension was transferred into plastic tubes. Measurement was started by an injection of 5 μM DCF-DA in the dark. After 30 min of incubation at 37 °C, and then ROS generation was determined by fluorescence spectrophotometer (F-450, Hitachi, Japan) at Ex = 485 and Em = 538 nm.

2.6. Nitrite assay

The cells were plated in 96-well plates and exposed with LPS (1 μg/ml) with/without various concentrations of SG (10–100 μg/ml) for 24 h. Nitrite was quantified colorimetrically after its reaction with Griess reagent [1% sufanilamide, 0.1% naphthalene diamine dichloride (NED), and 2% phosphoric acid] as described previously (Lee et al., 2005a). Briefly, the supernatants of cells were mixed with an equal volume of 1% sulfanilamide with 2% phosphoric acid at 15 min, and then NED dichloride at 15 min. The absorbance was measured with a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 540 nm within 30 min. The nitrite concentration was determined using sodium nitrite as a standard.

2.7. Western blotting

The cells were exposed to LPS (1 μg/ml) with/without various concentrations of SG (10–100 μg/ml) for 24 h. The cells were lysed with buffer [50 mM Tris (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 0.2% SDS, 1 mM PMFS, 10 μl/ml aprotinin, 1% igapel 630, 10 mM NaF, 0.5 mM EDTA, 0.1 mM EGTA and 0.5% sodium deoxycholate], and centrifuged at 15,000 × *g* for 15 min. Total proteins in the lysates were determined by the Bradford method (Lee et al., 2005a). Equal amount of proteins (40 μg) were separated on a SDS/10%-polyacrylamide gel, and then transferred to a nitrocellulose membrane (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA). Blots were blocked for 2 h at room temperature with 5% (w/v) non-fat dried milk in Tris-buffered saline [10 mM Tris (pH 8.0) and 150 mM NaCl] solution containing 0.05% Tween-20. The membrane was then incubated at room temperature with specific antibodies. Rabbit polyclonal antibodies against COX-2 (1:1000), iNOS (1:1000) and IκB (1:500), mouse polyclonal antibodies against $cPLA_2$ (1:500), pIκB (1:500) and β-actin (1:1000), and goat polyclonal antibodies against p50 (1:500) were used in this study. The β-actin levels were measured for the confirmation of equal amount of protein loading. Immunoreactive proteins were detected with the ECL Western blotting detection system (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA). The relative density of the protein band was quantified by densitometry using Electrophoresis Documentation and Analysis System 120 (Eastman Kodak Com., Rochester, NY).

2.8. Luciferase assay

The cells were transfected with pNF-κB-Luc plasmid (5× NF-κB; Stratagene, Cedar Creek, TX, USA) using a mixture of plasmid and lipofectAMINE PLUS (Invitrogen, Carlsbad, CA, USA) in OPTI-MEN solution (Gibco BRL, Grand Island, NY, USA) according to manufacture's specification. The control pCMV (Clontech Laboratories, Inc., Palo Alto, CA, USA) was co-transfected to monitor the transfection efficiency. After incubated for 24 h, the cells were co-treated with LPS (1 μg/ml) with/without various concentrations of SG (10–100 μg/ml) for 6 h. Total protein in the lysates was determined by the method of Bradford, and Luciferase activity was measured by using the luciferase assay kit according to the manufacture's instruction (WinGlow, Bad Wildbad, Germany), expressing as relative light units (RLU) per β-gal.

2.9. Nuclear extract and gel mobility shift assay

The cells were exposed with LPS (1 μg/ml) with/without various concentrations of SG (10–100 μg/ml) for 1 h and centrifuged at 3000 × *g* for 5 min. The pellets were resuspended in 400 l of cold buffer containing 10 mM HEPES, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol (DTT), and 0.2 mM phenylmethylsulfonyl fluoride (PMSF), and then centrifuged at 15,000 × *g* for 6 min to remove everything except the nuclear proteins. The pellets were resuspended in a second cold buffer containing 20 mM HEPES, 20% glycerol, 420 mM NaCl, 0.2 mM EDTA, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 0.2 mM PMSF. After centrifuged at 15,000 × *g* for 6 min, the supernatant contained the nuclear proteins. Total nuclear proteins in the lysates were determined by the Bradford method. The gel mobility shift assay was done using a slight modification method as previously described (Jung et al., 2003). Prior to the addition of labeled DNA probe, 10 μg of nuclear protein was incubated in binding buffer (10 μl) containing 10 mM Tris (pH 7.5), 100 mM NaCl, 1 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM EDTA, 4% glycerol and 0.05 mg/ml poly(dI–dC) for 10 min followed by another 20 min incubation with 100 μCi [γ-^{32}P] (NEN Life Science, Boston, MA, USA) ATP-labeled NF-κB oligonucleotide (Promega, Inc., Chatsworth, CA, USA) binding site at room temperature. The DNA-protein binding complex was mixed in loading buffer containing 25 mM Tris–HCl (pH 7.5), 4% glycerol, 0.02% bromophenol blue, then were electophoresed on 6% non-denatured polyacrylamide gel with 0.5× Tris–borate–EDTA (pH 8.0) at 150 V for 2 h. The gel was vacuum-dried and autoradiographed using X-ray film at −80 °C overnight. The relative densities of the bands were quantified by densitometry using Electrophoresis Documentation and Analysis System 120 (Eastman Kodak Com., Rochester, NY).

2.10. Immnocytochemistry and confocal laser microscope

Astrocytes (0.5 × 10^6 cells/cm^2) were cultured on a chamber slide (Lab-Tak II chamber slider system, Nalge Nunc Int., Naperville, IL, USA), fixed in 4% paraformaldehyde, membrane-permeabilized by exposure for 30 min to 0.1% Triton X-100 in phosphate-buffered saline, and placed in blocking serum (5% bovine serum albumin in phosphate-buffered saline) at room temperature. Cells were then exposed to primary goat polyclonal antibody for p50 or p65 (1:100 dilution) overnight at 4 °C, After three washes with ice-cold phosphate-buffered saline, followed by exposure with an anti-goat biotinylated secondary antibody (Molecular Probes, Eugene, USA) for 1 h at room temperature. Immunofluorescence images were acquired using a confocal laser scanning microscope (dual wavelength scan, MRC1024, Bio-Rad, Hercules, CA, USA) with a 360× oil immersion objective.

2.11. Statistics

Data were analyzed using one-way analysis of variance followed by Tukey's test as a post hoc test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Inhibitory effect of SG on LPS-induced cell death in astrocytes

In order to verify the inhibitory effect of SG on LPS-induced astrocyte cell death, we performed the cytotoxicity assay after 24 h culture by the WST-1 reduction assay in LPS-treated astrocytes. SG (10–100 μg/ml) significantly recovered 1 μg/ml LPS-induced inhibition of cell viability in a dose dependent manner (Fig. 1A). SG alone did not show any apparent cytotoxicity even after treatment with the highest concentrations (100 μg/ml, data not shown). This inhibitory effect of SG was enhanced by addition of glutathione. This significant inhibition of cytotoxicity was consistent with the inhibition on the LPS-induced cell death determined with DAPI staining (Fig. 1B). Interestingly, the highest dose of SG completely prevented LPS-induced cell death.

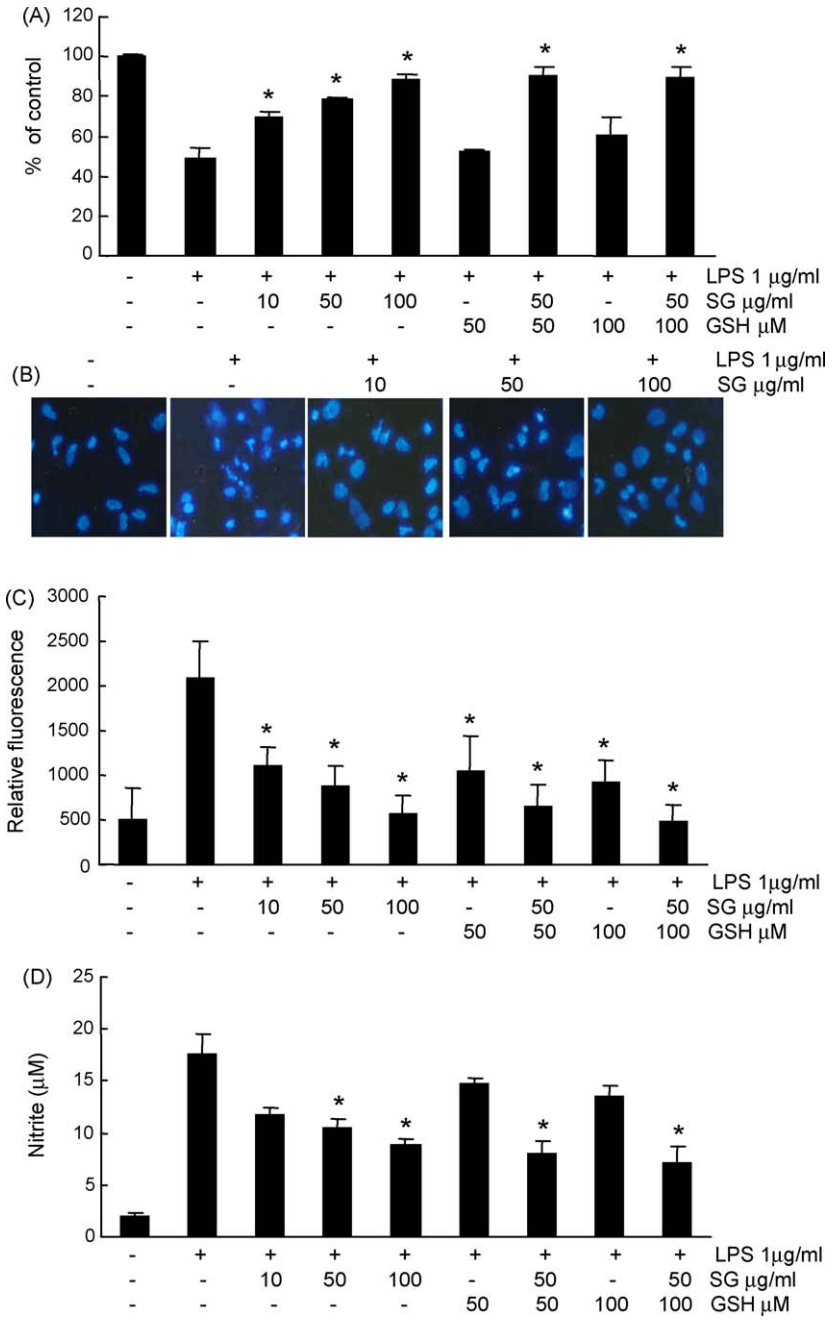

Fig. 1. Protective effect of SG on LPS-induced cytotoxicity, nitrite and reactive oxygen species generation in astrocytes. (A) The cell viability was determined with WST-1 assay. (B) The cell death was determined with DAPI staining method (B). Astrocytes were treated with 1 μg/ml LPS or co-treated with 10–100 μg/ml SG in the absence or presence of glutathione (GSH) for 24 h. Values are means ± S.D. from three experiments with duplicates. Asterisk (*) indicates significantly different from LPS treated alone ($p < 0.05$). (C) The reactive oxygen species level was determined by the method described in Section 2. Astrocytes were treated with 1 μg/ml LPS or co-treated with 10–100 μg/ml SG in the absence or presence of GSH for 24 h. Values are means ± S.D. from three experiments with triplicates. Asterisk (*) indicates significantly different from LPS treated alone (B) ($p < 0.05$). (D) The nitrite level was determined by the measurement of Griess reagent. Astrocytes was treated with 1 μg/ml LPS or co-treated with LPS and 10–100 μg/ml SG in the absence or presence of GSH for 24 h. Values are means ± S.D. from three experiments with triplicates. Asterisk (*) indicates significantly different from LPS treated alone ($p < 0.05$).

3.2. Inhibitory effect of SG on LPS-induced oxidative stress in astrocytes

Since cell death of astrocytes may be related with over release of ROS and NO, which can act as implicated contributors in the neuronal cell death, we determine whether SG inhibits ROS and NO production in LPS-treated astrocytes. The level of ROS and NO were determined in the culture supernatants. SG (10–100 μg/ml) significantly suppressed the ROS generation (Fig. 1C) as well as NO generation (Fig. 1D). Consistent with the recovery effect on LPS-induced cell growth inhibition, the inhibitory effect of SG on LPS-induced ROS and NO generation was enforced by the addition of glutathione.

3.3. Inhibitory effect of SG on LPS-induced inflammation genes expression in astrocytes

Since inflammation is implicated in the development or progression of AD, we examined the inhibitory effect of SG on LPS-induced inflammatory gene expressions such as $cPLA_2$, COX-2 and iNOS which are typical genes involving with inflammation in the brain. Since we found that the expression of these gene is still maintained for 24 h after treatment with LPS in the preliminary study (data not shown), and the inhibitory effect of cell death was determined at 24 h treatment, we assayed the expression of these genes after 24 h treatment. As shown in the most upper Western blotting panel (Fig. 2), SG (10–100 μg/ml) significantly inhibited LPS-induced $cPLA_2$, COX-2 and iNOS expressions. The densitometry analysis showed that this inhibition was dose dependent (Fig. 2A–C).

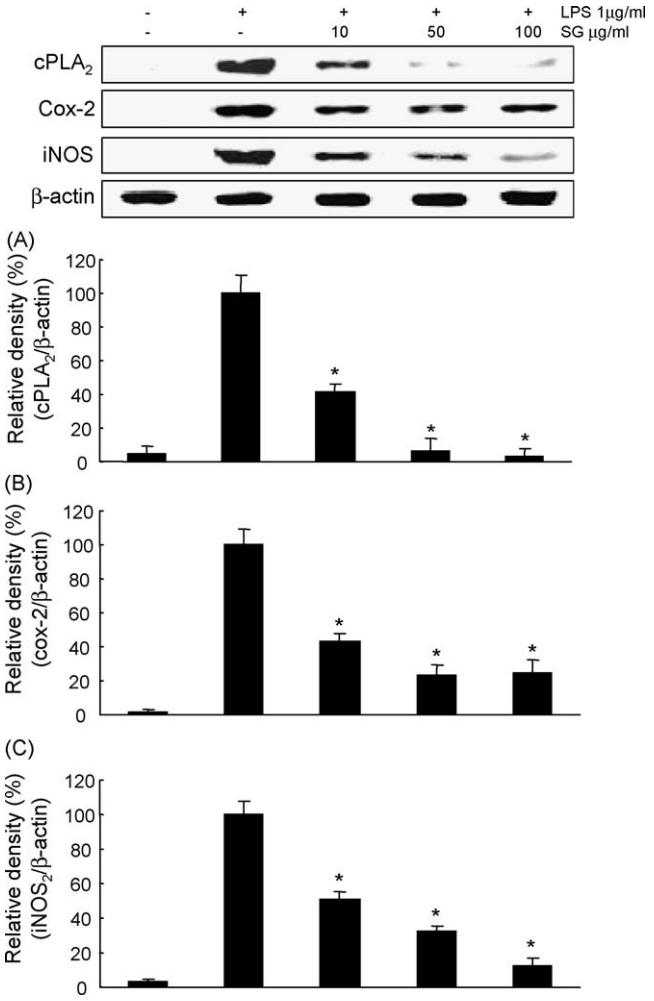

Fig. 2. Preventive effect of SG on the LPS-induced inflammatory gene expression in Astrocytes. Astrocytes were treated with 1 μg/ml LPS in the presence of indicated concentrations of SG (10–100 μg/ml) for 24 h. Immunoblots of cytoplasmic lysates from treated were probed with antibodies specific for $cPLA_2$ (A) COX-2 (B) and iNOS (C), respectively. Beta-actin levels were measured for the confirmation of equal amount of protein loading. Densitometric values are means from two separated experiments with triplicates. Asterisk (*) indicates significantly different from LPS treated alone ($p < 0.05$).

3.4. Inhibitory effect of SG on LPS-induced NF-κB activation in astrocytes

In order to investigate the inhibitory effect of SG on LPS-induced NF-κB transcriptional and DNA binding activities, we performed the NF-κB-dependant luciferase assay and EMSA. When astrocytes were co-treated LPS with/without a series of concentrations of SG (10–100 μg/ml), SG inhibited LPS-induced luciferase activity in a dose-dependent manner (Fig. 3A). Parallel with the inhibitory effect on the transcription activity, SG dose dependently inhibited NF-κB DNA binding activity (Fig. 3B). This binding activity was confirmed with supershift assay as well as competition assay (data not shown). Next, we investigated the mechanism of NF-κB inactivation by SG. Since translocation of NF-κB subunit into the nucleus is

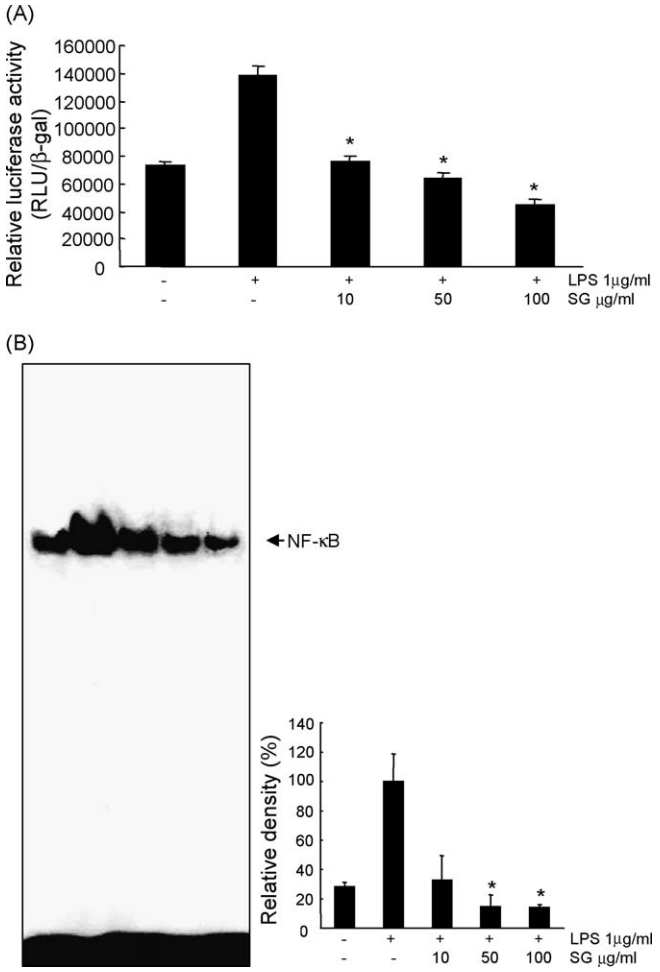

Fig. 3. Inhibitory effect of SG on LPS-induced NF-κB-dependent luciferase activity and NF-κB activation in Astrocytes. (A) NF-κB-dependent luciferase activity was determined by the method as described in Section 2. Astrocytes were treated with 1 μg/ml LPS or co-treated with LPS and 10–100 μg/ml SG for 6 h. Values are means from two separated experiments with triplicates. Asterisk (*) indicates significantly different from LPS treated alone ($p < 0.05$). (B) Astrocytes was treated with 1 μg/ml LPS in the presence of indicated concentrations of SG (10–100 μg/ml) for 1 h. Nuclear protein was used for EMSA to detect DNA binding activity of NF-κB, as described in Section 2. Densitometric values are means from two separated experiments with triplicates. Asterisk (*) indicates significantly different from LPS treated alone ($p < 0.05$).

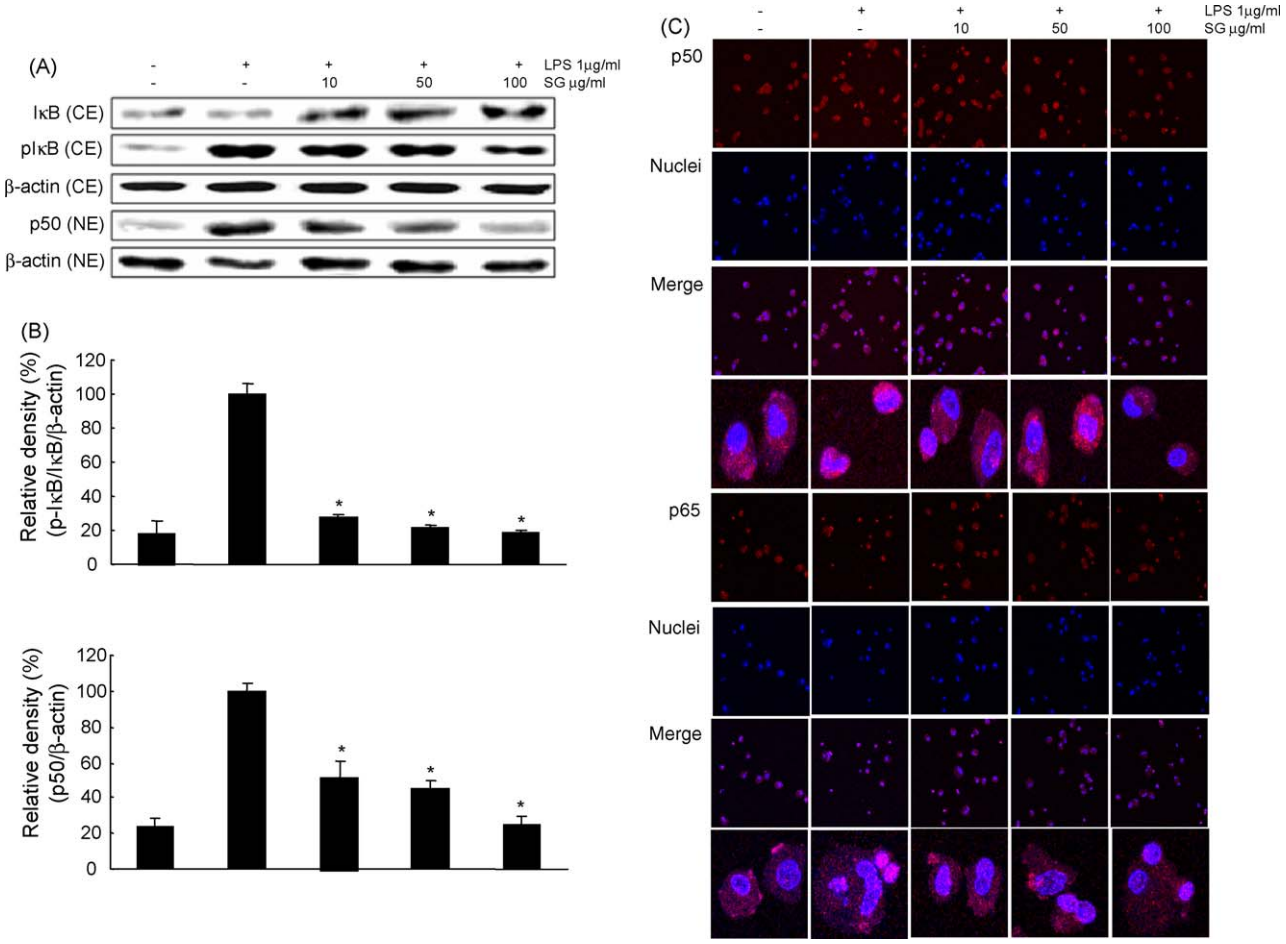

Fig. 4. Effect of SG on the levels of cytoplasmic unphosphorylated, phosphorylated IκBα and nuclear p50 in Astrocytes. Astrocytes were treated with 1 μg/ml LPS in the presence of indicated concentrations of SG (10–100 μg/ml) for 1 h. (A) Immunoblots of cytoplasmic and nuclear lysates from treated astrocytes were probed with antibodies specific for IκBα or phosphorylated IκBα and p50, respectively. NE, nuclear extract. CE, cytosolic extract. Beta-actin levels were measured for the confirmation of equal amount of protein loading. (B) Densitometric values are means from two separated experiments with triplicates. Asterisk (*) indicates significantly different from LPS treated alone ($p < 0.05$). (C) Confocal microscope observation of translocated p50 or p65 into nucleus. Immunofluorescence images were acquired using a confocal laser scanning microscope (360× or 1000×). Similar observation was detected in separated three experiments.

preceded by the phosphorylation and proteolytic degradation of IκB in cytosol, we examined the phosphorylation of IκB, and thereby translocation of p50 and p65 into nucleus. SG inhibited the LPS-induced phosphorylation of IκB accompanied with increase of IκB expression, and the expression of nuclear fraction of p50 in a dose-dependent manner (Fig. 4A and B). The inhibitory effect of p50 translocation into nucleus was confirmed by confocal microscopy observation, but the translocation of p65 was not significantly changed (Fig. 4C).

4. Discussion

Inflammation may play an important role in the pathogenesis of the neurodegenerative disorder such as AD (Irizarry and Hyman, 2001; McGeer and McGeer, 2003; Allan and Rothwell, 2003; Cacquevel et al., 2004; Tuppo and Arias, 2005). This disease characterized by deterioration of recognition ability and memory function (Ashe, 2001; Mattson, 2004). These deficits probably are due to the vulnerability of the brain cells to increased inflammation during disease processes. Among cells, astrocytes are reactivated by pro-inflammatory inducers such as LPS. The activated astrocytes induce neuro-inflammation and ultimately induce neuronal cell death in the AD-affected brain (McGeer and McGeer, 2003; Tuppo and Arias, 2005).

We previously found that subchronic administration of SG (3.75 and 7.5 mg/kg, p.o. for 3 weeks) significantly improved LPS and Aβ as well as scopolamine-induced recognitive performance evaluated by step through and water maze paradigm in animal model (Kim et al., 2003; Lee et al., 2005c). In addition, SG has also demonstrated to inhibit Aβ-induced PC12 cell death through anti-oxidative and anti-inflammatory mechanisms (Lee et al., 2005a). We have demonstrated that SG could recover LPS-induced astrocytes cell death via reduction of neuroinflammatory reaction, therefore, SG can increase neuronal cell survival in the brain against neuroinflammatory damages. However, the mechanism of preventive effect of SG on the LPS-induced astrocytes cell death is not clear. But, it is noteworthy that the activated astrocytes were detected near the amyloid plaque deposits and the expression of proinflammatory mediators (e.g., TNF-α, and

NO) were increased in the AD brain (Jain et al., 2002; McGeer and McGeer, 2003). It was also known that COX-2 and iNOS are expressed in the neurons of brain and the spinal cord under physiological circumstances and is overexpressed in several pathophysiological situations including AD (Acarin et al., 2002). These data indicate that inflammatory reactions in astrocytes play a definite role in the pathobiology of AD. Thus, inhibitory effect on inflammatory reaction of SG could protect from LPS-induced neuronal cell death. Results from many other investigations and epidemiological studies have demonstrated that anti-inflammatory medications targeting inactivation of astrocytes may have therapeutic potential in AD (McGeer and McGeer, 2003; Ayasolla et al., 2005; Tuppo and Arias, 2005). Taken together, the present data with the consideration of other data suggest that SG might have beneficial effects on the neuronal cell survival through reduced inflammatory reaction which may eventually prevent the formation of inflammatory complex of the neuronal plagues in AD.

Other possibility cannot be excluded as possible mechanisms. Ceramide is also a key mediator of apoptosis, and is involved in oxidative stress response and play an important role in controlling the fate of Astrocytes (Carracedo et al., 2004; Ohtani et al., 2004). Ceramide functions as an intracellular lipid mediator for a variety of cell functions, including apoptotic neuronal cell death (Okazaki et al., 1990; Obeid et al., 1993). The level of ceramide is upregulated by various types of stress conditions including hydrogen peroxide, and in neurological disease conditions such as AD and cerebral ischemia (Verheij et al., 1996; Kubota et al., 1996; Herr et al., 1999; Han et al., 2002), and can promote beta-peptide biogenesis (Cotrina and Nedergaard, 2002; Puglielli et al., 2003). Therefore, elevated ceramide could be possibly involved in LPS-induced astrocyte cell death. Thus, the inhibitory effect of SG on the generation of ROS and/or NO could be related with cell death through reduction of ceramide level. We are currently being investigated this issue.

Our results are similar to the finding by Jeng et al who demonstrated that sesamin inhibits LPS-induced cytokine production by suppression of NF-κB in the murine microglia and BV-2 cell line (Jengh and Singh, 2004). The mechanism of inhibitory effect of inflammatory gene expression was not clear. However, SG blocked LPS-induced activation of NF-κB which is a well known transcription factor to control the expression of inflammatory genes such as COX-2, $cPLA_2$ and iNOS. Therefore, the way SG inhibiting inflammatory reaction in astrocytes may be involved with the inactivation of NF-κB. When the activated NF-κB binds to NF-κB-responsive elements on the promoters of target genes in DNA binding domains, the target gene can be expressed. Our data showed that SG decreased the release of IκB, thus suppressed the nuclear translocation of p50. This data therefore indicates that one action mechanism of SG to prevent NF-κB is related with the inhibition of p50 translocation by inhibition of IκB release of NF-κB. The degree of inhibition of p50 translocation was much lesser than the inhibiting IκB phosphorylation suggests that SG may not fully inhibit IκB release, and other mechanism could be involved. IκB release can be inhibited by the upstream proteins of IκB such as IKK or 26s proteosome (Jain et al., 2002). Interestingly, there have been demonstrated that several IKK and 26s proteosome inhibitors have recently shown to be targets of NF-κB such as prostaglandin A_1, arsenite and parthenolide (Rossi et al., 1997; Hehner et al., 1999; Roussel and Barchowsky, 2000). It has not been reported whether SG has similar biological activity, but it is interesting to know that sesame lignan including sesaminol show proteolytic ability of membrane bound precursor form of transcription factor sterol regulatory element binding protein-1 (Ide et al., 2001). But, we observed that inhibitory effect on p65 translocation into nucleus was weak. This may be possible that SG may effectively inhibit the translocation of p50/p50 homodimer than p65/p65 homodimer or p65/p50 heterodimer. Other possibility that SG may be directly bound to the subunit (p65) which cannot be translocated into nucleus as in the case of many diterpenoid compounds which inhibit activation of NF-κB through inhibition of the translocation of one subunit of NF-κB (Leung et al., 2005). It has been demonstrated that agents inhibiting NF-κB can be useful in the treatment of the inflammatory disease including development and progression of AD (Jain et al., 2002). For example, Fiebich showed that the non-steroidal anti-inflammatory drug tepoxalin can be useful for the treatment of AD patient since it inhibits cytokine synthesis in astrocytes by preventing degradation of IκB-alpha (Fiebich et al., 1999). Vitamin E also suppresses microglial activation through inactivation of NF-κB which can be neuroprotective (Li et al., 2001).

In conclusion, the present data showed that SG attenuated the LPS-induced inflammatory gene expression through inactivation of NF-κB via prevention of p50 translocation, suggesting that SG may be as a useful agent for prevention of development or progression of inflammatory disease such as AD.

Acknowledgements

This work was supported by grant from the Korea Food Research Institute and by the Brain Korea 21 project in 2003–2005.

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