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Inhibitory Effects of Glycyrrhizae Radix and Its Active Component, Isoliquiritigenin, on Aβ(25-35)-induced Neurotoxicity in Cultured Rat Cortical Neurons

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This study investigated an ethanol extract from Glycyrrhizae radix (GR), the root of *Glycyrrhiza uralensis* (Leguminosae), for possible neuroprotective effects on neurotoxicity induced by amyloid β protein (Aβ) (25-35) in cultured rat cortical neurons. Exposure of cultured cortical neurons to 10 μM Aβ (25-35) for 36 h induced neuronal apoptotic death. GR (10-50 μg/mL) prevented the Aβ (25-35)-induced neuronal apoptotic death, as assessed by a MTT assay and Hoechst 33342 staining. Furthermore, GR decreased the expression of Bax and active caspase-3, proapoptotic proteins, and increased Bcl-2, an antiapoptotic protein. GR also significantly inhibited Aβ (25-35)-induced elevation of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and generation of reactive oxygen species (ROS) measured by fluorescent dyes. Isoliquiritigenin (1-20 μM), isolated from GR as an active component, inhibited Aβ (25-35)-induced neuronal apoptotic death, elevation of $[Ca^{2+}]_i$, ROS generation, and the change of apoptosis-associated proteins in cultured cortical neurons, suggesting that the neuroprotective effect of GR may be, at least partly, attributable to this compound. These results suggest that GR and isoliquiritigenin prevent Aβ (25-35)-induced neuronal apoptotic death by interfering with the increases of $[Ca^{2+}]_i$ and ROS, and GR may have a possible therapeutic role for preventing the progression of neurodegenerative disease such as Alzheimer's disease.

Key words: Glycyrrhizae radix, Isoliquiritigenin, Cultured neurons, Amyloid β protein, Neuroprotection, Alzheimer's disease

INTRODUCTION

Alzheimer's disease (AD), the most common form of the senile dementia, is a progressive neurodegenerative disorder that deprives the patient of memory and eventually leads to death. Although the pathological mechanism of AD is uncertain, it is characterized by its two histopathological hallmarks, senile plaque and neurofibrillary tangles (Yankner, 1996) Amyloid β protein (Aβ), a major constituent of senile plaque, is a 39-43 amino acid fragment derived from amyloid precursor protein (Golde et al., 1992) and is thought to be a significant factor in developing AD. Many studies indicated that Aβ neurotoxicity might involve oxidative stress (Behl et al., 1994), excessive increases in intracellular calcium level ($[Ca^{2+}]_i$) (Mattson et al., 1992), excitotoxicity induced by glutamate release and a resultant apoptotic neuronal death (Ohyagi et al., 2000). Therefore, the blockades of these pathways are main interest for prevention and treatment of AD.

Glycyrrhiza species (Leguminosae) have long been recognized as one of the most famous medicinal plants in traditional oriental medicine, and widely used due to its diverse pharmacological properties including vasorelaxant (Yu and Kuo, 1995), anti-allergic (Shin et al., 2007), antitumor (Chung et al., 2001), anti-inflammatory (Shin et al., 2008), and anti-oxidant effects (Tang et al., 2004). There is growing evidence for Glycyrrhizae radix (GR) (GR), the root of *Glycyrrhiza uralensis*, with beneficial effects in brains such as

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antidepressant (Wang et al., 2008) and memory enhancing effects (Dhingra et al., 2004; Ahn et al., 2006) and protection of ischemic brain damage in animal models (Hwang et al., 2006). Flavonoids found in numerous fruits, vegetables, grains, and plants have been demonstrated to be effective in preventing stroke (Keli et al., 1996), coronary heart diseases (Tijburg et al., 1997), and cancer (Block et al., 1992) because of their potent free radical scavenging properties. There are also considerable researches on specific flavonoid constituents such as liquiritigenin, isoliquiritigenin and grabridin isolated from GR of their anti-dementia and anti-ischemic effects in animal models (Zhan and Yang, 2006; Cui et al., 2008; Sun et al., 2010) as well as antioxidant (Kumar et al., 2007) and anti-inflammatory effects (Kim et al., 2008). The aim of the present study was to investigate the neuroprotective effect of an ethanol extract of GR against Aβ (25-35)-induced neuronal death and the underlying mechanism in primarily cultured rat cortical neurons. Additionally, we identified isoliquiritigenin (ISL) as active component contributing to the neuroprotective effect of GR against Aβ (25-35)-induced neurotoxicity.

MATERIALS AND METHODS

Plant material, preparation, and isolation of ISL

GR was purchased from Daegu Oriental Pharm Co. at Daegu, Korea and identified by one of the authors (Dr. Kung-sik Song). A voucher specimen (KNUNPC-GR-06-001) was deposited at Natural Products Chemistry Laboratory, Kyungpook National University, Daegu, Korea. The dried GR (250 g) was refluxed with 20 L of 95% ethanol for 3 h and the extract was filtered through filter paper (Advantec MFS). The filtrate was concentrated to dryness under reduced pressure with a rotary evaporator. The ethanolic extract (41.92 g) was suspended in distilled water and successively partitioned with CH_2Cl_2. Organic solvent fraction was collected and concentrated by a rotary evaporator. The active CH_2Cl_2 soluble fraction (8.86 g) was subjected to open column chromatography with silica gel [3.8 × 50 cm, *n*-hexane-ethylacetate = 30:1~1:1] to give 5 fractions (Fr. 1-5). Compound **1** (6 mg) was isolated from Fr. 3 (1.20 g) by re-chromatography with a silica gel column (4 × 28 cm, *n*-hexane-acetone = 8:1~1:1). The fractions and isolated compounds were monitored by thin layer chromatography (TLC) using several organic solvents and visualized by 10% sulfuric acid. For structural determination, ^{1}H- and ^{13}C- nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance Digital 400 NMR spectrometer at 400 and 100 MHz, respectively. Proton chemical shifts were referenced using tetramethylsilane (TMS) as an internal reference, and carbon chemical shifts were referenced to the solvent. Chemical shifts (ä) are expressed in ppm relative to TMS. Solvents were removed below 40°C under reduced pressure with a rotary evaporator (EYELA). TLC was performed on a pre-coated silica gel plate (Kieselgel 60F254, Merck) and silica gel column chromatography was carried out using Kieselgel 60 (Merck).

Compound 1: yellow powder. ^{1}H-NMR (400 MHz, CD_3OD): 7.96 (1H, d, J = 8.9 Hz, H-6'), 7.78 (1H, d, J = 15.3 Hz, H-β), 7.61 (2H, d, J = 8.6 Hz, H-α), 7.60 (1H, d, J = 15.3 Hz, H-2 and H-6), 6.83 (2H, d, J = 8.6 Hz, H-3 and H-5), 6.40 (1H, dd, J = 2.4, 8.9 Hz, H-5'), 6.28 (1H, d, J = 2.4 Hz, H-3'). ^{13}C-NMR (100 MHz, CD_3OD): 193.7 (C=O), 167.6 (C-4'), 166.5 (C-2'), 161.7 (C-4), 145.8 (C-β), 133.5 (C-6'), 131.9 (C-2 and C-6), 128.0 (C-1), 118.5 (C-α), 117.0 (C-3 and C-5), 114.8 (C-1'), 109.3 (C-5'), 103.9 (C-3'). These were good accordance with NMR data of ISL in the reference (Zheng et al., 2008). Compound **1** was determined as ISL. The structure of the ISL is shown in Fig. 1.

Experimental animals

Pregnant Sprague-Dawley (SD) rats were purchased from KOATECH co. Ltd for primary culture of cerebral cortical neurons. Rats were housed in environmentally controlled rooms at 22 ± 2°C, with a relative humidity of 55 ± 5%, a 12 h light/dark cycle and food and water *ad libitum*. The procedures involving experimental animals complied with the regulations for the care and use of laboratory animals of the animal ethical committee of Chungbuk National University.

Induction of neurotoxicity in primary cultures of rat cortical neurons

Primary cortical neuron cultures were prepared using embryonic day 15 to 16 SD rat fetuses, as previously described (Ban et al., 2006). Neurotoxicity experiments were performed on neurons after 3-4 days in culture. Cultured neurons were treated with 10 μM Aβ (25-35) (Bachem) in serum-free DMEM (Sigma) at 37°C for 36 h (unless otherwise indicated) to produce

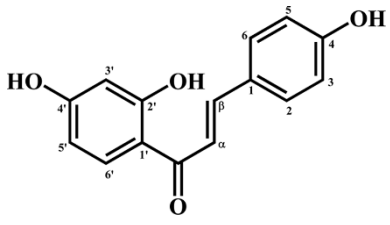

Fig. 1. Chemical structure of ISL isolated from GR.

neurotoxicity. An Aβ (25-35) stock solution of 2 mM was prepared in sterile distilled water, stored at –20°C, and incubated for more than 2 days at 37°C to aggregate before use. GR and ISL were dissolved in DMSO at concentrations of 50 mg/mL and 20 mM, respectively, and further diluted in experimental buffers. The final concentration of DMSO was 0.1%, which did not affect cell viability. For each experiment, GR and ISL were applied 15 min prior to treatment with 10 μM Aβ (25-35). They were also present in the medium during Aβ (25-35) incubation.

Measurements of Aβ (25-35)-induced neuronal death and intracellular biochemical changes

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma) assay and Hoechst 33342 (Molecular Probes) staining were performed to measure neuronal death 36 h after exposure of cultured neurons to 10 μM Aβ (25-35), as previously described (Ban et al., 2006). Changes in $[Ca^{2+}]_i$ were measured with Fluo-4 AM (Molecular Probes), a calcium-sensitive fluorescent dye, using a laser scanning confocal microscope (LSM 510, Carl Zeiss) with 488-nm excitation argon laser and 515-nm longpass emission filters. The microfluorescence of 2',7'-dichlorofluorescein diacetate (H_2DCF-DA; Molecular Probes), and a laser scanning confocal microscope (MRC1024ES, Biorad) with 488-nm excitation and 510-nm emission filters were used to monitor the generation of ROS in neurons treated with 10 μM Aβ (25-35) for 24 h.

Western blot

Neurons treated with 10 μM Aβ (25-35) for 36 h on dishes were lysed with RIPA buffer containing 150 mM NaCl, 1 mM Na-EDTA, protease inhibitor cocktail, and 50 mM Tris-HCl, pH 7.4 to extract protein. The amount of protein was measured by the Bradford method (Bradford, 1976). Approximately 50 μg of total protein were loaded on 12.5% SDS-PAGE and transferred to a PVDF membrane (Perkin Elmer Co.). The membranes were incubated with primary antibodies against Bcl-2, BAX, procaspase-3, active caspase-3 and β-actin (1:1000) (Millipore Inc.), followed by horseradish-peroxidase conjugated anti-rabbit (1:1500) secondary antibodies (Assay Designs Inc). Protein expression was detected with an enhanced chemiluminescence detection reagent (Santa Cruz Biotechnology Inc.). Images were quantified using image analysis software (a freely available application in the public domain for image analysis and process, developed and maintained by Wayne Rasband at the Research Services Branch, National Institutes of Health).

Statistical analysis

Data are expressed as mean ± S.E.M. and statistical significance was assessed by one-way analysis of variance (ANOVA) and Tukey's test. $P < 0.05$ was considered significant.

RESULTS

GR and ISL inhibit A β (25-35)-induced neuronal cell death

It has been demonstrated that Aβ (25-35) reflects the biologically active region of full length Aβ (Pike et al., 1995). In our previous studies, it was demonstrated that Aβ (25-35) within a concentration range of 5-20 μM produced a concentration-dependent reduction of cell viability in cultured cortical neuron (Ban et al., 2006). Therefore, we used 10 μM Aβ (25-35) to induce neuronal cell damage in the present experiment. When cortical neurons were exposed to 10 μM Aβ (25-35) for 36 h, MTT reduction rate decreased to 66.7 ± 1.9% of the control level (untreated cells). Pretreatment of GR (10, 25 and 50 μg/mL) concentration-dependently reduced the Aβ (25-35)-induced decrease of MTT reduction showing 90.7 ± 3.6% with 50 μg/mL (Fig. 2). Liquiritigenin and ISL were isolated from ethanol extract of GR and used in this study to determine which components of GR exhibit neuroprotective effect in cultured cortical neurons. Of these two compounds, only ISL reduced neuronal death induced by Aβ (25-35) showing 85.9 ± 2.3% with 20 μM (Fig. 2).

Hoechst 33342 staining in Aβ (25-35)-treated neurons was performed to manifest apoptotic neuronal death.

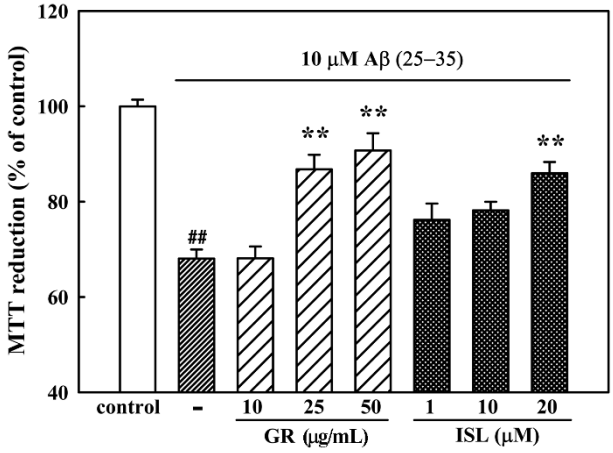

Fig. 2. Effects of GR and ISL on Aβ (25-35)-induced neuronal death in cultured cortical neurons. Neuronal cell death was measured using the MTT assay. The MTT absorbance from untreated cells was normalized to 100%. Results are expressed as mean ± S.E.M. of data obtained from 5 independent experiments. $^{\#\#}p < 0.01$ *vs* control; $^{**}p < 0.01$ *vs* 10 μM Aβ (25-35).

Morphological feature of apoptosis such as chromatin condensation and nuclear fragmentation were observed in neurons treated with 10 μM Aβ (25-35), whereas the control culture had round blue nuclei of viable neurons (Fig. 3A). The proportion of apoptotic neurons was calculated as shown in Fig. 3B. Treatment of neurons with 10 μM Aβ (25-35) induced apoptosis in 36.4 ± 2.6% of cultured cortical neurons, compared with 10.3 ± 1.4% of control cultures. GR (10, 25 and 50 μg/mL) significantly decreased the Aβ (25-35)-induced apoptotic cell death, showing apoptosis of 18.1 ± 2.0, 19.3 ± 2.6 and 18.6 ± 3.0% of total population of cultured cortical neurons, respectively. ISL also reduced apoptosis showing 22.6 ± 4.0% with 20 μM (Fig. 3B).

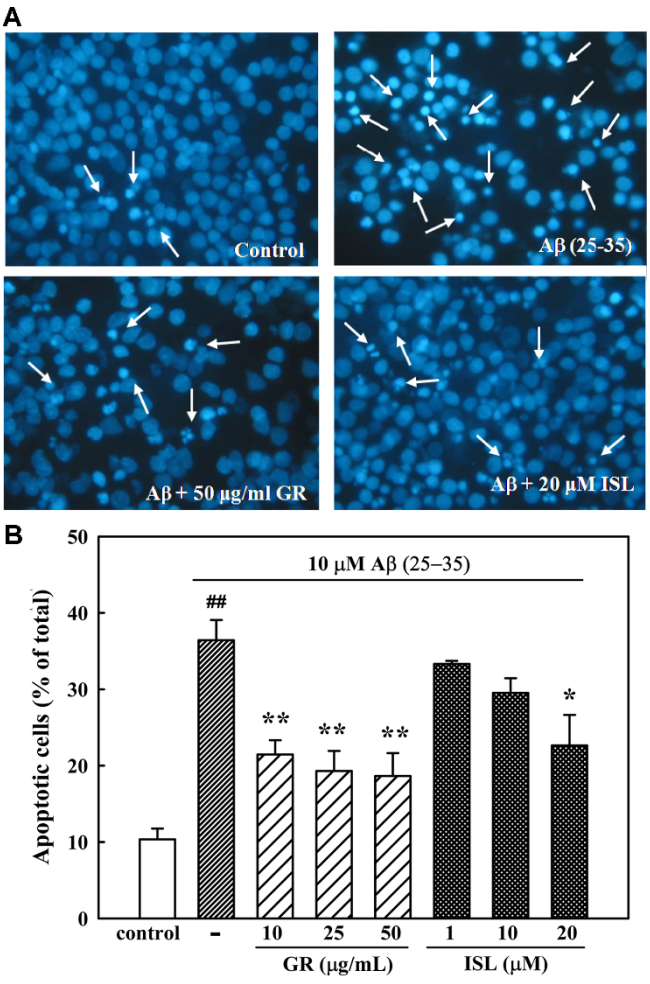

Fig. 3. Effects of GR and ISL on Aβ (25-35)-induced apoptosis of cultured cortical neurons. Apoptotic cells measured by Hoechst 33342 staining were counted in 5 to 6 fields per well. (**A**) Representative photomicrographs of cultured neurons showing Aβ (25-35)-induced apoptosis. The arrows indicate fluorescence typical for apoptotic nuclei. (**B**) The values represent the apoptotic cells as a percentage of the total number of cells and expressed as mean ± S.E.M. of data obtained from 4 independent experiments. $^{\#\#}p < 0.01$ *vs* control; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* 10 μM Aβ (25-35).

GR and ISL inhibit Aβ (25-35)-induced pro- and anti-apoptotic protein expression change

Expression of anti-apoptotic protein, Bcl-2, was

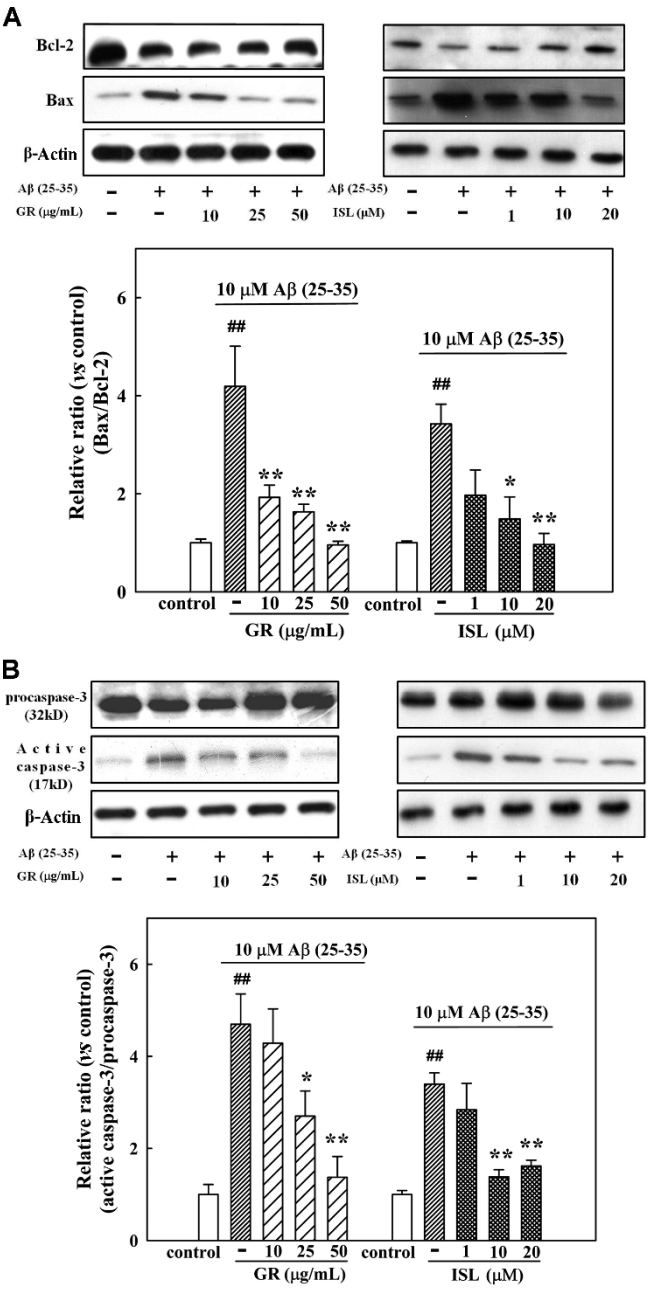

Fig. 4. Effects of GR and ISL on Aβ (25-35)-induced expression of apoptosis-associated proteins in cultured cortical neurons. Bcl-2 and Bax (**A**) and procaspase-3 and active caspase-3 (**B**) were determined by Western blotting. Representative blots obtained from four similar results are shown with each bar graph. Relative densities of Bax/Bcl-2 (**A**) and active caspase-3/procaspase-3 (**B**) were measured by densitometric assay using Image J software. $^{\#\#}p < 0.01$ *vs* control; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* 10 μM Aβ (25-35).

decreased by Aβ (25-35) treatment, while GR and ISL prevented the Bcl-2 decrease induced by Aβ (25-35). In addition, overexpression of pro-apoptotic protein, Bax, induced by Aβ (25-35) was inhibited by GR and ISL treatment (Fig. 4A). We studied the activation of caspase-3, the apoptotic executor, in cultured neurons represented by the ratio of active caspase/total caspase-3 (Fig. 4B). Results show a great increase in activation of caspase-3 by treatment of 10 μM Aβ (25-35) for 36 h. This activation was significantly inhibited by GR and ISL.

GR and ISL inhibit Aβ (25-35)-induced elevation of $[Ca^{2+}]_i$

It has been postulated in many studies that the increase of $[Ca^{2+}]_i$ is involved in Aβ-induced neurotoxicity (Mattson et al., 1992). As shown in Fig. 5, $[Ca^{2+}]_i$ rapidly increased in response to treatment with 10 μM Aβ (25-35) and then showed a slow and gradual decrease over 10 min. In contrast, pretreatment with GR (50 μg/mL) and ISL (20 μM) significantly inhibited the increase of $[Ca^{2+}]_i$ induced by 10 μM Aβ (25-35) throughout the measurement period. GR and ISL did not affect basal $[Ca^{2+}]_i$ (data not shown).

GR and ISL inhibit Aβ (25-35)-induced ROS generation

Free radical injury is one of the most common and important way inducing neuronal cell death. To make it clear whether GR and ISL prevent Aβ (25-35)-induced oxidative damages in cultured neurons, the accumulation of ROS was measured after the exposure of neurons to 10 μM Aβ (25-35) for 24 h. In H_2DCF-DA-loaded cortical neurons, the fluorescence intensity of 10 μM Aβ (25-35)-treated neurons increased approximately 3.7-fold to 224.7 ± 8.9 compared with control neurons of 60.9 ± 5.9, indicating the generation of ROS. GR (25 and 50 μg/mL) and ISL (1, 10 and 20 μM) significantly blocked the ROS generation induced by Aβ (25-35) (Fig. 6).

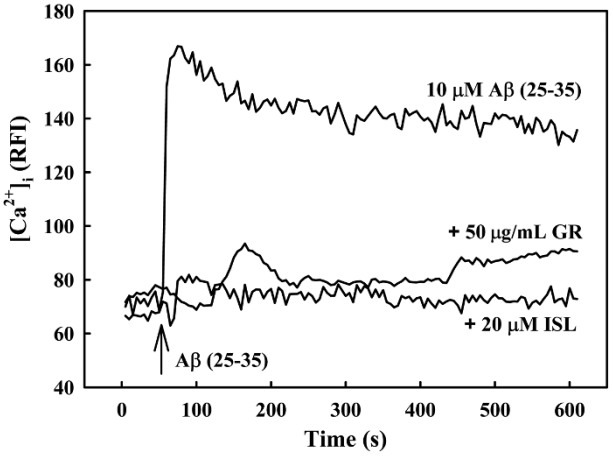

Fig. 5. Effects of GR and ISL on Aβ (25-35)-induced elevation of $[Ca^{2+}]_i$ in cultured cortical neurons. $[Ca^{2+}]_i$ was monitored using Fluo-4 AM dye and a confocal laser scanning microscope. All images were processed to analyze changes in $[Ca^{2+}]_i$ at the single cell level. Results are expressed as the relative fluorescence intensity (RFI). Each trace shows a single cell that is representative of at least 3 independent experiments.

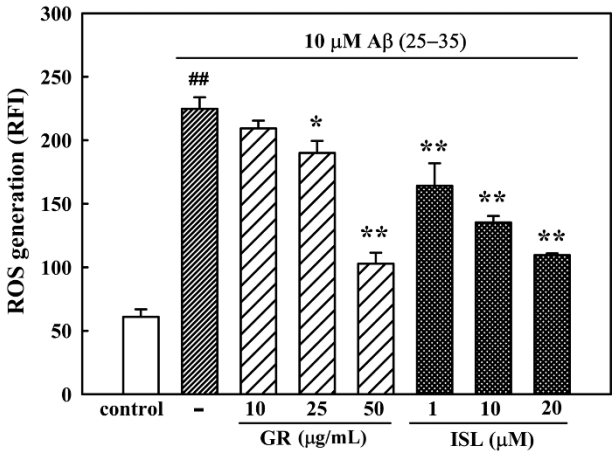

Fig. 6. Effects of GR and ISL on Aβ (25-35)-induced ROS generation in cultured cortical neurons. ROS was monitored using H_2DCF-DA dye and a confocal laser scanning microscope. Results are expressed as mean ± S.E.M. of RFI obtained from 4 independent experiments. $^{\#\#}p < 0.01$ *vs* control; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* 10 μM Aβ (25-35).

DISCUSSION

In the present study, we showed that GR which is the most frequently used in traditional medicines and its active compound ISL ameliorated the neuronal apoptotic death induced by Aβ (25-35) by preventing $[Ca^{2+}]_i$ increase and ROS generation in primarily cultured rat cortical neurons.

It is well known that Aβ induces complex neurotoxic insults such as the disruption of $[Ca^{2+}]_i$ homeostasis (Mattson et al., 1992), oxidative stress (Behl et al., 1994), hyperphosphorylation of Tau (Yankner, 1996), and overexpression of pro-apoptotic proteins (Hong et al., 2003). The present study also clarified the involvement of $[Ca^{2+}]_i$ increase, ROS generation, and apoptotic neuronal death in Aβ (25-35)-induced neurotoxicity. In our previous studies (Cho et al., 2009; Jeong et al., 2010), all of these effects induced by Aβ (25-35) were blocked by MK-801, an N-methyl-D-aspartate (NMDA) antagonist; verapamil, a L-type Ca^{2+} channel blocker; and N^G-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, in cultured neurons.

These results suggest that NMDA glutamate receptor activation, Ca^{2+} influx through L-type voltage dependent Ca^{2+} channel (L-VDCC) and ROS generation are implicated in Aβ-induced neuronal apoptotic death. Of these many factors, Ca^{2+} influx via L-VDCC is considered as a primary insult after Aβ treatment in cultured neurons, because blockade of this channel or Ca^{2+} chelation prevents other consequences (Ueda et al., 1997). Although physiological concentration of $[Ca^{2+}]_i$ plays pivotal roles in neuronal growth and differentiation (Missiaen et al., 2000), excessively increased $[Ca^{2+}]_i$ perturbs neuronal self-regulation system. In the present study, GR completely inhibited a rapid increase of $[Ca^{2+}]_i$ caused immediately after the treatment with Aβ (25-35). GR also inhibited ROS generation and neuronal cell death induced by Aβ (25-35) in cortical neuronal culture. These findings indicate that sustained inhibition on Aβ (25-35)-induced $[Ca^{2+}]_i$ elevation by GR prevented ROS generation and neuronal death.

Many researchers revealed that Aβ (25-35) leads to mitochondrial dysfunction (Pereira et al., 1998; Kim et al., 2002). Mitochondria uptakes cytosolic Ca^{2+} to maintain $[Ca^{2+}]_i$ homeostasis (Castaldo et al., 2009). Calcium overload in mitochondria leads to opening of mitochondrial permeability transition pore and inhibition of the activity of mitochondrial respiratory chain complex followed by decrease in mitochondrial oxygen consumption, mitochondrial membrane depolarization, and ATP depletion (Pereira et al., 1998). In this way, Ca^{2+} accumulation in cytosol contributes to ROS generation from its major source, mitochondria. Furthermore, ROS generation conversely induces secondary Ca^{2+} surge by mediating membrane damage, consequently it accelerates the neuronal demises (Mailly et al., 1999). GR contains antioxidant flavonoids such as ISL. Therefore, it also can be suggested that the GR could inhibit the delayed $[Ca^{2+}]_i$ increase through suppression of ROS generation, and resultantly ameliorated Aβ (25-35)-induced neuronal death.

Apoptosis morphologically characterized by nuclear fragmentation and chromatin condensation increases in neurodegenerative disease such as AD. These typical features of apoptosis were expressed in cultured cortical neurons exposed to Aβ (25-35), which were reduced by GR in the present study. Aβ (25-35) directly mediates extrinsic apoptotic pathway by interacting with death receptors like tumor necrosis factor receptor-1 and Fas receptor (Vaisid et al., 2009). Aβ (25-35) also mediates intrinsic apoptotic pathway by damaging to mitochondria which are significantly related to $[Ca^{2+}]_i$ homeostasis and ROS generation (Kuperstein and Yavin, 2003). Both apoptotic pathway are regulated by Bcl-2 family members (e.g. Bcl-2, Bax, Bak, Bad), and converge onto activation of caspase-3 which is the apoptotic executor (Hockenbery et al., 1993). Endoplasmic reticulum stress induced by elevated $[Ca^{2+}]_i$ also contributes to activation of caspase-3 (Costa et al., 2010). Cultured cortical neurons exposed to Aβ (25-35) exhibited increased expression of active caspase-3 in the current study. GR reduced the increase of active caspase-3 expression, although it was not identified which pathway is involved in activation of caspase-3. Furthermore, overexpression of pro-apoptotic protein Bax and decreased expression of anti-apoptotic protein Bcl-2 by Aβ (25-35) were also inhibited by GR. It is thus concluded that GR could prevent the Aβ (25-35)-induced neuronal death by interfering with the increase of $[Ca^{2+}]_i$ firstly and then ROS generation, followed by the inhibition on the change of apoptosis-related proteins expression in cultured neurons. It has been reported that aqueous extract of GR inhibited cognitive deficits induced by the intracerebroventricular administration of Aβ (25-35) in mice and protected against Aβ (25-35)-induced neuronal death in PC12 cells, a neuron-like dividing cell line (Ahn et al., 2006, 2010), whereas we used primarily cultured rat cortical neurons. They focused on anti-oxidative effect of GR. We used an ethanol extract of GR and isolated ISL from the ethanol extract. Furthermore, the concentration of ~1.0 mg/mL used for the neuroprotection in PC12 cells was ~20 times higher than the highest concentration of 50 μg/mL used in the present study. These results may explain that there are considerable differences of active components between water and ethanol extract of GR. The present results demonstrated a novel pharmacological activity of an ethanol extract of GR in primarily cultured neurons.

It is well known that GR contains many licorice flavonoids including liquiritin, isoliquiritin, liquiritigenin, and ISL. Especially ISL was reported to have the neuroprotective effect against transient middle cerebral occlusion-induced focal cerebral ischemia in rats with potent antioxidant activity (Zhan and Yang, 2006). In this study, ISL reduced Aβ-induced $[Ca^{2+}]_i$ increase, ROS generation and, in result, attenuated neuronal apoptotic death in primary cultures of rat cortical neurons. ISL reduced Aβ (25-35)-induced increase of active caspase-3 expression. Overexpression of pro-apoptotic protein Bax and decrease of anti-apoptotic protein Bcl-2 by Aβ (25-35) were also inhibited by ISL. Therefore, the inhibition of Aβ (25-35)-induced neuronal damage by GR in cultures may partially result from the beneficial effects of ISL.

In conclusion, this study demonstrated that an ethanol extract of GR and its active compound ISL pro-

tected against Aβ (25-35)-induced neuronal damage in cultured rat cortical neurons. This neuroprotective effect of GR in *in vitro* might be responsible for the protective effect against Aβ (25-35)-induced learning and memory deficits in *in vivo* model and GR could be a promising agent for the treatment of neurodegenerative disease such as AD.

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