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Protein Kinase C-mediated Neuroprotective Action of (−)-epigallocatechin-3-gallate against $A\beta_{1-42}$-induced Apoptotic Cell Death in SH-SY5Y Neuroblastoma Cells

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The neurotoxicity of amyloid β (Aβ) is associated with an increased production of reactive oxygen species and apoptosis, and it has been implicated in the development of Alzheimer's disease. While (-)-epigallocatechin-3-gallate (EGCG) suppresses Aβ-induced apoptosis, the mechanisms underlying this process have yet to be completely clarified. This study was designed to investigate whether EGCG plays a neuroprotective role by activating cell survival system such as protein kinase C (PKC), extracellular-signal-related kinase (ERK), c-Jun N-terminal kinase (JNK), and anti-apoptotic and pro-apoptotic genes in SH-SY5Y human neuroblastoma cells. One μM $A\beta_{1-42}$ decreased cell viability, which was correlated with increased DNA fragmentation evidenced by DAPI staining. Pre-treatment of SH-SY5Y neuroblastoma cells with EGCG (1 μM) significantly attenuated $A\beta_{1-42}$-induced cytotoxicity. Potential cell signaling candidates involved in this neuroprotective effects were further examined. EGCG restored the reduced PKC, ERK, and JNK activities caused by $A\beta_{1-42}$ toxicity. In addition, gene expression analysis revealed that EGCG prevented both the $A\beta_{1-42}$-induced expression of a pro-apoptotic gene mRNA, Bad and Bax, and the decrease of an anti-apoptotic gene mRNA, Bcl-2 and Bcl-xl. These results suggest that the neuroprotective mechanism of EGCG against $A\beta_{1-42}$-induced apoptotic cell death includes stimulation of PKC, ERK, and JNK, and modulation of cell survival and death genes.

Key Words: Aβ, Alzheimer's disease, Apoptosis, (−)-epigallocatechin-3-gallate, PKC

INTRODUCTION

Alzheimer's disease (AD) is a common neurodegenerative disease characterized by progressive impairment of cognitive function and loss of memory in association with widespread neuronal death (Barinaga, 1995; Yamazaki et al, 1996). Neuronal loss in AD is accompanied by the deposition of amyloid beta protein (Aβ) in senile plaques and the presence of neurofibrillary tangles (Braak et al, 1998). Generally, Aβ has been shown to induce oxidative stress both in vitro and in vivo (Yatin et al, 1999; Butterfield and Lauderback, 2002; Drake et al, 2003). Aβ hydrophobic polypeptide consisting of 39-43 amino acid residues, is proteolytically produced by β- and γ-secretases from a single transmembrane polypeptide collectively referred to as the amyloid precursor protein (Sinha and Lieberburg, 1999; Sultana et al, 2005). Although the processing of amyloidogenic amyloid precursor protein (APP) occurs even in normal conditions, diverse genetic defects which cause inherited AD have been shown to augment it, leading to the hypothesis that increased Aβ production and/or failure of Aβ clearance mechanisms might be key pathogenetic steps in AD (Selkoe, 2002). It has been demonstrated that Aβ is toxic directly to cultured neuron (Behl et al, 1994), supporting the hypothesis that Aβ is involved in the neurodegeneration associated with AD, but the mechanisms involved in the Aβ-mediated neurotoxicity remain unclear (Choi et al, 2001). The two main constituents of amyloid plaques, $A\beta_{42}$ and $A\beta_{40}$, can not only exert pro-apoptotic effects when extracellularly applied to cultured neurons, but also they enhance the intracellular production of Aβ peptides (Copani et al, 1999; Lu et al, 2003; Pizzi et al, 2005; Valerio et al, 2006).

Green tea consumption is considered as a source of dietary constituents endowed with biological and pharmacological activities with potential benefits to human health (Graham, 1992). Most of the experimental and epidemiological studies on green tea effects have been targeted on its possible cardiovascular, anti-inflammatory, and anticarcinogenic effects, which have been linked to the anitoxidant/pro-oxidant properties of its polyphenol constituents (Pan

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ABBREVIATIONS: Aβ, beta amyloid; AD, Alzheimer's disease; APP, amyloid precursor protein; EGCG, (−)-epigallocatechin gallate; PKC, Protein Kinase C; ERK, extracellular-signal-related kinase; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.

et al, 2000; Mandel et al, 2004). Among the tea catechins, (-)-epigallocatechin-3-gallate (EGCG) is the major constituent, accounting for more that 10% of dry weight of the extract, followed by (-)-epigallocatechin (EGC) > (-)-epicatechin (EC) ≥ (-)-epicatechin-3-gallate (ECG) (Mandel et al, 2004). EGCG has been studied extensively due to its diverse physiological and pharmacological properties, including hypolipidemic, anti-inflammatory, anti-microbial, anti-oxidative, and anti-carcinogenic activities (Ahmad et al, 1997; Masuda et al, 2001; Levites et al, 2002).

PKC is a well established neuronal cell survival factor, also participating in cell growth and differentiation (Ruvolo et al, 1998; Jiffar et al, 2004). Indeed, activation of PKC by the grape flavonoid resveratrol protects hippocampal neurons against $A\beta$-toxicity (Han et al, 2004), and treatment of human cells with EGCG induces a specific translocation of PKC to the membrane (Kim et al, 2004). Therefore, phosphorylative activation of PKC by EGCG appears to be responsible for the protective effects against 6-OHDA-induced cell death in SH-SY5Y (Levites et al, 2002) and for the neurorescue effect against long-term growth factors withdrawal in PC12 cells (Reznichenko et al, 2005). Recently, we found that EGCG can potently inhibit $A\beta_{1-42}$-induced cell death, however, the mechanisms underlying this process have yet to be completely clarified. In this study, we investigated whether EGCG can suppress $A\beta$-induced cell death, and whether EGCG has the potential to modulate the expression of anti-/pro-apoptotic genes and PKC activation in human SH-SY5Y neuroblastoma cells.

METHODS

Cell and culture conditions

SH-SY5Y human neuroblastoma cells were cultured in DMEM (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (FBS, Hyclone) and 1% penicillin-streptomycin (Hyclone). SH-SY5Y neuroblastoma cells were differentiated for 5 days in the presence of 10 μM retinoic acid (RA, Sigma Chemical Co., St. Louis, MO., USA). Treatment of the cells with RA for this time stops cell division and induces differentiation, as judged by the elaboration of neurites of length ≥ 2 respective somal diameters in more than 90% of cells. Cells were grown to approximately 80% confluence in a 37°C humidified incubator with 5% CO_2 and 95% air environment, and then harvested in trypsin containing EDTA (Hyclone). $A\beta_{1-42}$ (American peptide Inc., CA, USA) was initially dissolved in a small volume of dimethyl sulfoxide (DMSO, Sigma Chemical Co.), diluted to a final stock concentration of 200 μM in serum free medium and aggregated at 37°C before use.

(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

MTT (Sigma Chemical Co.) is absorbed into cell, and formazan is then formed by the action of mitochondrial succinate dehydrogenase. Accumulation of formazan reflects the activity of mitochondria directly and the cell viability indirectly. Thus, the cells were plated at a density of 1×10^4 cells/well in 96-well plates (Nalge Nunc International, Rochester, NY, USA), and then pre-incubated with EGCG (1 μM) (Sigma Chemical Co.) for 2 h before the addition of 1 μM $A\beta_{1-42}$. Twenty four hours after exposure of cells to $A\beta_{1-42}$, a total 20 μl of 5 mg/ml MTT were added into each well and the cells were incubated for 3 h at 37°C in the dark. A supernatant solution was removed from each well, and 100 μl of DMSO were then added to each well. Optical density (OD) at 570 nm was evaluated on the ELISA plate reader (Molecular Devices, Sunnyvale, CA). All results were calibrated for OD measured in the same conditioned well without cell culture.

Nuclear staining with 4',6-diamidino-2-phenyindole (DAPI) to assess apoptosis

In order to determine whether $A\beta_{1-42}$ induces apoptosis, DAPI (Sigma Chemical Co.) staining was performed as described previously (Yim et al, 2000). Briefly, SH-SY5Y neuroblastoma cells were cultured on 6-well plates at cell density of 5×10^4 cells/well. After treatment with EGCG and $A\beta_{1-42}$, cells were washed twice with phosphate-buffered saline (PBS) and fixed by incubation in 4% PFA for 15 min. Following a second washing in PBS, cells were permeabilized in 0.5% Triton X-100 (Sigma Chemical Co.) for 5 min, incubated in 1 μg/ml DAPI solution for 30 min in the dark, and then observed through a fluorescence microscope (Fluorescent Microscopy, Olympus IX50, Olympus America, Melville, NY, USA). The number of apoptotic cells, which coincided with morphological criteria of apoptosis, such as DNA fragmentation, nuclear condensation and segmentation, were identified in the 400 cells randomly.

Reverse transcription-polymerase chain reaction (RT-PCR) to examine of pro- and anti-apoptotic gene expressions

The cells were cultured on 6-well plates in DMEM containing 10% FBS, EGCG was added for 2 h, and then $A\beta_{1-42}$ was added for 6 h. At the end of incubation, the medium was removed, and total RNA was isolated using TriReagent (Molecular Research Center, Inc., Cincinnati, OH, USA) isolation reagent. And 300 μl of CIAA (chloroform : isoamyl alcohol = 49 : 1 at 50 ml tube, light sensitive, Sigma Chemical Co.) solution were added into each 1ml of Trizol reagent and vortexed for 15 sec. Samples were then centrifuged at 4°C and 13,200 rpm for 20 min, and top clear layer was transferred into a new tube. After same volume of isopropanol (Sigma Chemical Co.) was added and mixed by inversion, tubes were kept at −70°C for 1 h and centrifuged at 4°C and 13,200 rpm for 30 min. The supernatant was removed, and the pellet was washed with

Table 1. Sequence of RT-PCR primer and product size

Gene	Sequence (5'-3')	Size (bp)
Bcl-2	sense : CGACTTCGCCGAGATGTCCAGGCAG antisense : GACCCACGGATAGACCCGGTGTTCA	388
Bcl-xl	sense : GAATCTTATCTTGGCTTTGGA antisense : GTAGAGTGGATGGTCAGTGT	799
Bad	sense : TCCCAGAGTTTGAGCCGAGT antisense : ATGTGGAGCGAAGGTCACTG	471
Bax	sense : TGCTTCAGGGTTTCCAGG antisense : ACCACTGTGACCTGCTCCAGAA	476
GAPDH	sense : CATGACCACAGTCCATGCCATCACT antisense : TGAGGTCCACCACCCTGTTGCTGTA	461

800 μl of 70% ethanol (Honeywell, Burdick & Jackson, Muskegon, MI, USA) and spun down for 5 min. The supernatant was then poured out, and the pellet was washed with 800 μl of 100% ethanol and spun down for 5 min. Finally, the pellet was dried, dissolved in RNase-free water (Gibco BRL, Grand Island, NY, USA), and then 1 μg of total RNA was reverse-transcribed into first-strand cDNA using oligo-dT primer (Bioneer Co., Daejeon, Korea). Reverse transcription was carried out with M-MLV reverse transcriptase (Gibco BRL) for 90 min at 42°C.

The cDNA was amplified by 25-35 cycles of PCR (Takara Bio Inc., Shiga, Japan) using Ex-Taq polymerase (Takara Bio Inc.). The primers used were chosen and checked for specificity using a basic BLAST search: Bcl-2, Bcl-xl, Bad, Bax, and GAPDH (Table 1). RT-PCR products were separated electrophoretically on 2% agarose gels.

Western blot analysis

SH-SY5Y neuroblastoma cells were cultured on 6-well plates in DMEM containing 10% FBS, and EGCG was administered for 2 h followed by A$\beta_{1\text{-}42}$ for 6 h. The phosphorylated and non-phosphorylated forms of PKC were observed by Western blot analysis. Briefly, 3×10^5 cells were washed twice in cold PBS, lysed in a lysis buffer [50 mM Tris pH 7.5, 100 mM NaCl, 1 mM EDTA, 1% NP-40, 10% glycerol, Leupeptin (50 μg/ml), Aprotinin (50 μg/ml), 0.2 M PMSF, 1 mM Na_3VO_4, 1 mM NaF], and incubated for 10 min on ice. Cell lysates were centrifuged at 4°C and 13,200 rpm for 10 min. Protein concentration in the cell lysates was determined by Quant-iT™ assay kit (Molecular probes). An equal amount (10 mg) of protein for each sample was resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membranes were blocked with 1% skim milk in Tris buffer saline containing 0.1% Tween-20 (TBST), and sequentially incubated with specific antibodies for PKC (1 : 2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-PKC (1 : 1000, Santa Cruz Biotechnology), JNK (1 : 2000, Santa Cruz Biotechnology), p-JNK (1 : 1000, Santa Cruz Biotechnology), ERK2 (1 : 2000, Santa Cruz Biotechnology), and p-ERK1/2 (1 : 1000 Santa Cruz Biotechnology). The membranes were washed with TBST and then processed using HRP-conjugated anti-rabbit antibodies, followed by ECL detection (Santa Cruz Biotechnology).

Statistics

Levels of RNA and protein were quantified by measuring the optical density of each band using computer-assisted densitometry (NIH Image analysis program, version 1.61). All values are expressed as mean±SEM. The one-way ANOVA test (Bonferroni post hoc comparison) was used to analyze the differences between groups, with $p<0.05$ being considered significant.

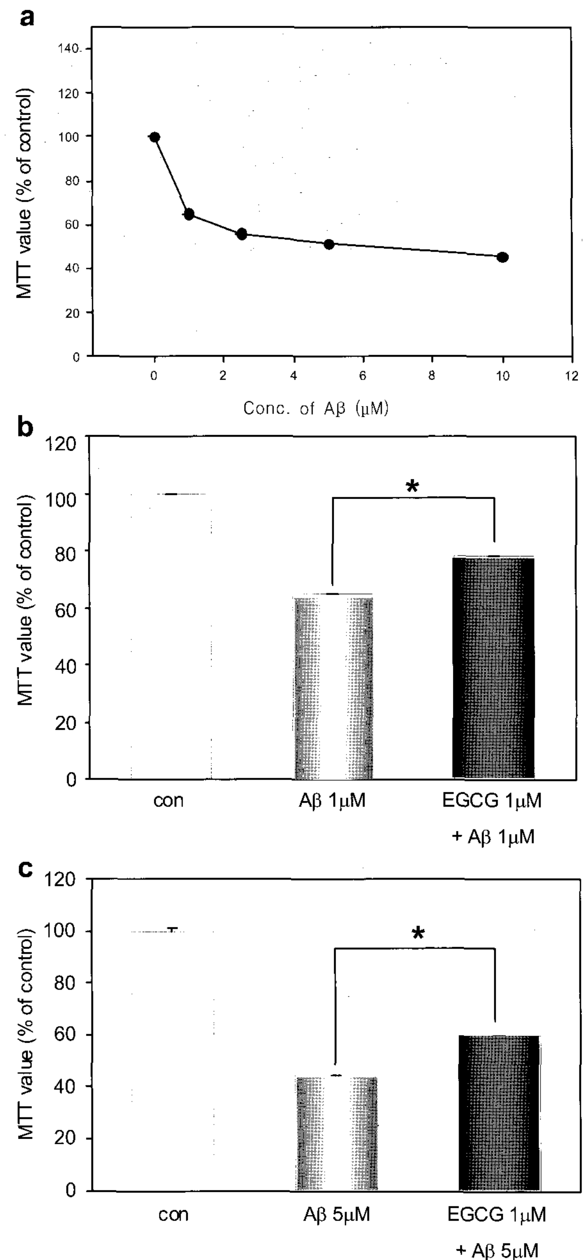

Fig. 1. A$\beta_{1\text{-}42}$ neurotoxicity and protection by EGCG in SH-SY5Y neuroblastoma cells as measured by MTT reduction assay (a, b, and c). The data represent mean±SE of three independent experiments, with each point done in quadruplicate. (a) Viability of neuroblastoma cells decreases with increasing A$\beta_{1\text{-}42}$ concentration. (b) Relative to untreated cells, treatment of cells with 1 μM A$\beta_{1\text{-}42}$ showed a significant decrease in MTT reduction. Such changes were not observed for 2 h of pretreatment with 1 μM EGCG, followed by 1 μM A$\beta_{1\text{-}42}$. (c) Same as b, but 5 μM A$\beta_{1\text{-}42}$ was used. *indicates significantly different from cells treated with A$\beta_{1\text{-}42}$ ($p<0.05$; Student's *t* test).

RESULTS

Neuroprotective effect of EGCG against A$\beta_{1\text{-}42}$-induced neurotoxicity

A$\beta_{1\text{-}42}$-induced cytotoxicity was evaluated by determining the percentage of MTT reduction after incubation of SH-SY5Y human neuroblastoma cells for 24 h with 1, 2.5, 5, 10 μM A$\beta_{1\text{-}42}$ as described in methods. After exposure to increasing concentration of A$\beta_{1\text{-}42}$, there were significant

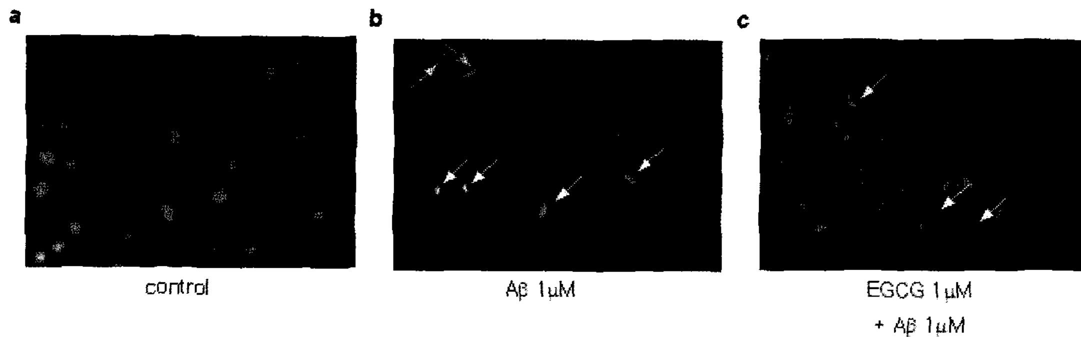

Fig. 2. Detection of apoptosis-like cell death in SH-SY5Y neuroblastoma cells by DAPI staining. Treated and untreated cells were stained with DAPI and examined for condensation and fragmentation. (a) Control neurons have a round of an ovoid shape. (b) Cells treated with only 1 μM Aβ_{1-42} show condensation and fragmentation of the nuclei, suggesting that they are undergoing apoptosis-like cell death. (c) Number of apoptotic cells decreased with pretreatment of 1 μM EGCG. Arrow indicates apoptosis-like cell death.

decreases in the percentage of survival cells in a concentration-dependent manner, ranging from 65.08±0.43% at 1 μM to 45.36±0.16% at 10 μM (Fig. 1a). Because the viability of SH-SY5Y neuroblastoma cells was about 60~70% at 1 μM, 1 μM was selected for subsequent experiments. To investigate the neuroprotective effects of EGCG, SH-SY5Y neuroblastoma cells were pretreated with 1 μM EGCG for 2 h, and then exposed to 1 and 5 μM Aβ_{1-42} for 24 h. The percentage of survival cells was increased from 65.08± 0.43% to 78.50±0.19% at 1 μM Aβ_{1-42} treatment and from 44.22±0.14% to 60.02±0.13% at 5 μM Aβ_{1-42} treatment (Fig. 1b and 1c).

To detect apoptosis-like cell death in SH-SY5Y neuroblastoma cells, cell nuclei were stained with DAPI. The number of apoptotic cells was significantly increased at 1 μM Aβ_{1-42} (Fig. 2b) compared with that in the control group (Fig. 2a), however, markedly decreased, when SH-SY5Y neuroblastoma cells were pretreated with 1 μM EGCG (Fig. 2c).

Effect of EGCG on Aβ_{1-42}-induced alterations of pro- and anti-apoptotic genes

To further investigate neuroprotective action of EGCG, apoptosis and cell survival related gene expression analysis was performed. Thus, SH-SY5Y neuroblastoma cells were treated with Aβ_{1-42} (1 μM), EGCG (1 μM) prior to Aβ_{1-42} (1 μM), or EGCG (1 μM) alone for 6 hr. The treatment of 1 μM Aβ_{1-42} decreased the mRNA expressions of Bcl-xl and Bcl-2 genes whereas increased those of Bad and Bax genes. However, in SH-SY5Y neuroblastoma cells pretreated with EGCG prior to Aβ_{1-42}, the mRNA levels of Bcl-xl and Bcl-2 genes were increased compared to those of Aβ_{1-42} alone, while the Bad and Bax mRNA levels were decreased compared to those of Aβ_{1-42} alone (Fig. 3). Also, EGCG did not affect the expression of antiapoptotic genes, Bcl-xl and Bcl-2, while it downregulated the expressions of pro-apoptotic genes, Bax and Bad. Treatment with Aβ_{1-42} and EGCG did not affect the expression of GAPDH mRNA in SH-SY5Y neuroblastoma cells, suggesting that the preventive effects of EGCG by pro- and anti-apoptotic genes were not due to generalized activation of these cells. These results indicate a protective effect of EGCG on variety of survival factors, which may contribute to the counteraction of Aβ_{1-42} toxicity.

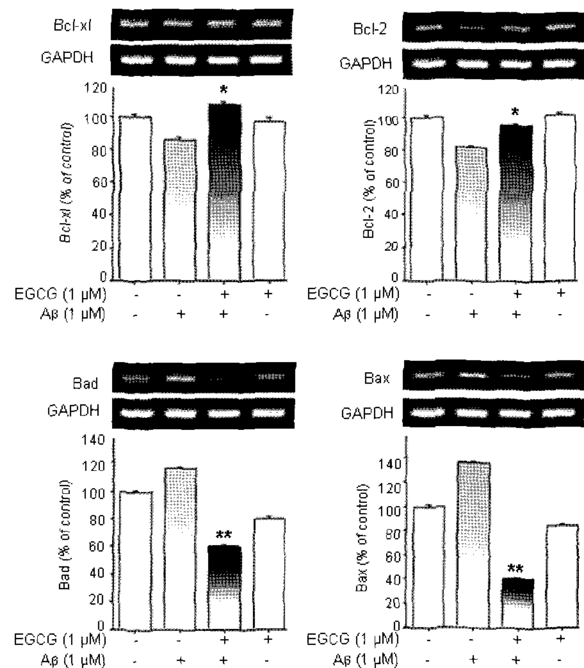

Fig. 3. Modulation of anti- and pro-apoptotic gene expressions by EGCG and Aβ_{1-42}. SH-SY5Y neuroblastoma cells were treated with EGCG (1 μM), Aβ_{1-42} (1 μM), or their combination for 6 h. Cellular RNA from each treatment was extracted, and mRNA expressions for Bcl-2, Bcl-xl, Bad, Bax, and GAPDH were analyzed by RT-PCR. Quantitative data of SH-SY5Y neuroblastoma cells are expressed as percentage of simultaneously assayed control group's value. *$p<0.05$, **$p<0.001$ compared with Aβ_{1-42}.

Involvement of PKC, ERK, and JNK in the neuroprotective effect of EGCG against Aβ_{1-42}-induced toxicity

Neuronal damage, emerging from oxidative stress, has been reported to involve regulation of cell signaling molecules, which are associated with cell death and cell survival (Singer et al, 1999; Schroeter et al, 2001). Green tea polyphenol fraction and EGCG have been shown to modulate PKC, ERK, and JNK pathways (Yu et al, 1997;

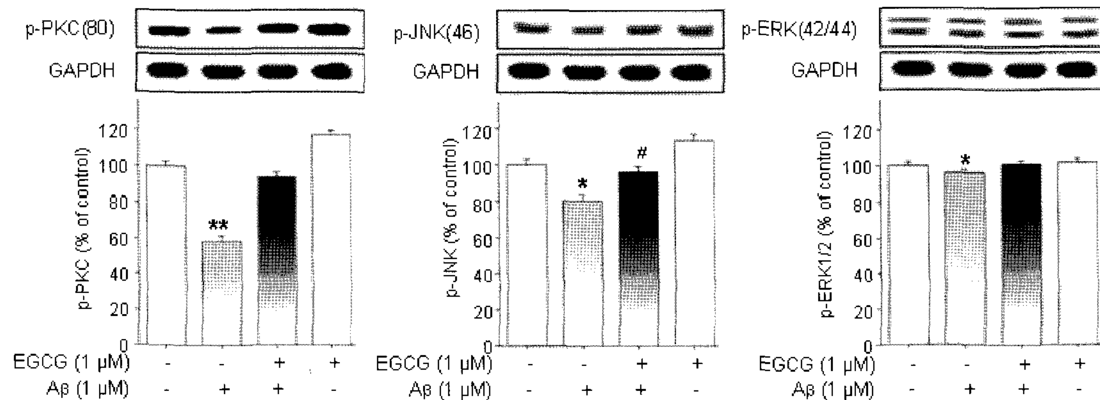

Fig. 4. Effects of EGCG and $A\beta_{1\text{-}42}$ on PKC, JNK, and ERK immunoreactivities in SH-SY5Y neuroblastoma cells. Cells were treated as described in Fig. 3. Cellular proteins from each treatment group were extracted with lysis buffer. *p<0.05, **p< 0.001 compared with control; #p<0.05 compared with $A\beta_{1\text{-}42}$.

Levites et al, 2002). There, we further examined the involvement of PKC and ERK and JNK pathways in the neuroprotective action of EGCG. SH-SY5Y neuroblastoma cells were treated with EGCG (1 μM), $A\beta_{1\text{-}42}$ (1 μM), or their combination for 6 h. Cellular proteins from each groups were extracted with cell lysis buffer and protein expression was analyzed by Western blotting. The immunoreactivity (IR) of PKC phosphorylation was found to decrease only in the group treated with 1 μM $A\beta_{1\text{-}42}$ alone and to significantly increase by pretreatment with EGCG (Fig. 4). Also, treatment of with SH-SY5Y neuroblastoma cells 1 μM $A\beta_{1\text{-}42}$ decreased the IRs of JNK and ERK phosphorylations at 6 h. In addition, treatment with EGCG prior to 1 μM $A\beta_{1\text{-}42}$ increased the IR levels of JNK and ERK phosphorylations, suggesting that activation of PKC, JNK, and ERK is the main cascade mediating the neuroprotective effect of EGCG against $A\beta_{1\text{-}42}$ toxicity.

DISCUSSION

This study was designed to investigate the effects of EGCG against $A\beta_{1\text{-}42}$-induced neuronal cell death at the cellular and molecular levels. Detailed investigation on the mechanism involved in this neuroprotective effect shows the restoration of reduced PKC, JNK, and ERK phosphorylation levels induced by $A\beta_{1\text{-}42}$, as well as modulation of proapoptotic and antiapoptotic-related genes. We suggest that activation of these signaling molecules is a part of the neuroprotective mechanisms that are activated by EGCG to counteract death signals in SH-SY5Y human neuroblastoma cells. However, the possible contribution of radical scavenging action of EGCG which has been well established cannot be ruled out. The neuroprotective effect of EGCG may apparently be due to its potent antioxidant and iron-chelating properties, as $A\beta$ neurotoxicity has been reported to be mediated by free radicals and attenuated by antioxidants and free radical scavengers (Tremblay et al, 1999; Pan et al, 2000).

Our results are in accordance with recent studies showing that green tea extract could prevent $A\beta$-induced neurotoxicity in cultured hippocampal cells (Choi et al, 2001; Bastianetto et al, 2006). Further support comes from a study, where EGCG was shown to exhibit protective effect against $A\beta$-induced damage in SH-SY5Y human neuroblastoma cells and PC12 cells, via PKC-dependent pathway (Levites et al, 2003). Furthermore, a recent report shows that treatment of human cells with EGCG induces a specific translocation of PKC to the membrane (Kim et al, 2004). More direct evidence implicating PKC in EGCG mechanistic action has come from a recent study employing solid-state nuclear magnetic resonance, showing that EGCG interacts with the head group region of the phospholipids within lipid bilayers from liposomes (Kumazawa et al, 2004). Consistent with these findings, recent animal studies have shown that two weeks of EGCG consumption (2 mg/kg) led to a highly significant up-regulation of PKC isoform in mice striatum (Mandel et al, 2004) and to a significant increase of PKC isoenzymes in membrane and cytosolic fractions of mice hippocampus (Levite et al, 2003). Several studies suggest that PKC expression in neurons is coupled with preservation of cell survival and prerequisite for neuroprotection against several exogenous insults (Vianna et al, 2000; Dempsey et al, 2000; Maher, 2001). Furthermore, brain-derived neurotrophic factor prevented the decline of PKC activity, resulting from N-methyl-D-aspartate-induced excitotoxicity, in primary rat cortical neurons (Tremblay et al, 1999), and activation of PKC by phorbol 12-myristate 13-acetate protected the hippocampal cell line from glutamate toxicity, and this effect was mediated through activation of ERK1/2 and JNK (Maher, 2001). Moreover, ERK1/2 and JNK activities in neurodegeneration induced were regulated by reactive oxygen species (Xia et al, 1995), activation of ERK1/2 is associated with pro-survival signaling (Bonni et al, 1999) and survival of primary cortical neurons intoxicated with glutamate (Singer et al, 1999), and apoptosis of PC 12 cells was induced by nerve growth factor withdrawal (Xia et al, 1995). Therefore in an attempt to characterize the signaling pathways involved in $A\beta_{1\text{-}42}$-induced cell death and in neuroprotection by EGCG, we examined the activity of ERK and JNK. When SH-SY5Y neuroblastoma cells pretreated with EGCG were exposed to $A\beta_{1\text{-}42}$, not only immunoreactivity of phosphorylated PKC, but also those of phosphorylated JNK and ERK were increased.

Previous studies have shown that EGCG decreased the expression of pro-apoptotic genes (e.g. Bax, Bad, Fas, and caspase 1, 3, 6, and 9) and restored the activities of PKC

and ERK following an exposure to 6-OHDA (Levites et al, 2002). We also showed the effect of EGCG on the pro- and anti-apoptotic gene expressions in $A\beta_{1-42}$-induced neurotoxicity model. Our results show that EGCG could protect $A\beta_{1-42}$-induced apoptotic cell death not only through the decrease of mRNA expression of pro-apoptotic genes (Bax and Bad), but also through the increase of those of anti-apoptotic genes (Bcl-2 and Bcl-xl). Bax has been known to homodimerize or heterodimerize with Bcl-2 or Bcl-xl (Yang et al, 1995), and these interactions have been shown to determine the susceptibility of a cell to a death signal. Decreased Bax mRNA levels, as a result of EGCG treatment in this study, may contribute to an increase in the ratio of Bcl-2 or Bcl-xl to Bax, thus providing potential neuroprotective/antiapoptotic mechanism of EGCG.

In conclusion, this study provides a neuroprotective mechanistic action of EGCG against $A\beta_{1-42}$-induced toxicity by stimulating PKC, ERK, and JNK and modulating the expression of cell survival and death genes.

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