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Green tea polyphenol (–)-epigallocatechin-3-gallate prevents ultraviolet-induced apoptosis in PC12 cells

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Green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG) is a potent antioxidant with protective effects against neurotoxicity. However, it is currently unclear whether EGCG protects neuronal cells against radiation-induced damage. Therefore, the objective of this study was to investigate the effects of EGCG on ultraviolet (UV)-induced oxidative stress and apoptosis in PC12 cells. The effects of UV irradiation included apoptotic cell death, which was associated with DNA fragmentation, reactive oxygen species (ROS) production, enhanced caspase-3 and caspase-9 activity, and poly (ADP-ribose) polymerase cleavage. UV irradiation also increased the Bax/Bcl-2 ratio and mitochondrial pathway-associated cytochrome c expression. However, pretreatment with EGCG before UV exposure markedly decreased UV-induced DNA fragmentation and ROS production. Furthermore, the UV irradiation-induced increase in Bax/Bcl-2 ratio, cytochrome c upregulation, and caspase-3 and caspase-9 activation were each ameliorated by EGCG pretreatment. Additionally, EGCG suppressed UV-induced phosphorylation of p38 and rescued UV-downregulated phosphorylation of ERK. Taken together, these results suggest that EGCG prevents UV irradiation-induced apoptosis in PC12 cells by scavenging ROS and inhibiting the mitochondrial pathways known to play a crucial role in apoptosis. In addition, EGCG inhibits UV-induced apoptosis via JNK inactivation and ERK activation in PC12 cells. Thus, EGCG represents a potential neuroprotective agent that could be applied to prevent neuronal cell death induced by UV irradiation.

Keywords: Apoptosis, Epigallcatechin, PC12 cells, Radiation

Introduction

Ultraviolet (UV) radiation present in natural sunlight and therapeutic artificial devices is a key human health concern. UV irradiation has been implicated in the development of various cancers in humans, including skin cancer, basal and squamous

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cell carcinoma, and cutaneous malignant melanoma [1,2]. UV can be subdivided into UVA (315–400 nm), UVB (280–315 nm), and UVC (200–280 nm) components. Natural solar UVA and UVB radiation can penetrate the atmosphere and reach the Earth's surface in amounts that have a significant impact on the biosphere. Although UVC cannot reach the Earth's surface, it can be generated by artificial sources such as photocuring devices and germicidal lamps emitting UV irradiation. The harmful radiation, including UVB and UVC primarily, induces DNA damage at the maximal absorption peak of 245–290 nm [3]. Cell damage induced by UV radiation is associated with cell-cycle arrest, cell death, mutation, neoplastic transformation, and immunosuppression, leading to photoaging and photocarcinogenesis [4]. UV irradiation induces apoptosis of neuronal cells. Thus, UV irradiation has been used as a tool to determine the underlying mechanism of apoptosis [5–7]. UV irradiation induces cell death via a variety of cellular mechanisms, including induction of nuclear DNA damage, activation of cell surface death receptors, formation of reactive oxygen species (ROS) accompanied by mitochondrial dysfunction, and release of pro-apoptotic factors such as cytochrome *c* [8–10].

Apoptosis, also known as programmed cell death, is a normal developmental response to physiological stimulus [11,12]. In general, apoptosis is driven by the activation of a family of cysteine proteases called caspases, which cleave a set of critical cellular proteins to initiate apoptotic cell death. These caspases are expressed as proenzymes and activated by upstream stimuli [13]. Mammalian caspases comprise at least 14 members. Caspases associated with apoptosis can be further subdivided into initiator caspases (-2, -8, -9, and -10) and effector caspases (-3, -6, and -7) [14]. Besides these caspases, members of Bcl-2 protein family also play a critical role in the regulation of apoptosis. The Bcl-2 family of proteins are functionally divided into anti-apoptotic (Bcl-2, Bcl-xL, Bcl-W, Mcl-1, and A1) and pro-apoptotic (Bax, Bcl-1s, Bid, Bad, Bim, and Bik) types [15]. Anti-apoptotic Bcl-2 and its homologs are anchored in the mitochondrial membrane to stabilize the transmembrane potential [16]. Mitochondria-mediated apoptotic pathway leads to the initiation of cell death by altering the mitochondrial permeability, resulting in pore formation, followed by release of cytochrome *c* and oxygen free radicals into the cytosol [16].

Tea polyphenols are natural plant flavonoids found in leaves and stems of tea plant. Green tea polyphenols exhibit a variety of pharmacological properties, including anti-inflammatory, anticarcinogenic, and antioxidant effects [17–19]. Green tea polyphenols comprise (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin, (–)-epicatechin, (+)-gallocatechin, and catechin. Among these polyphenols, EGCG is a major component exhibiting various biological activities [20–22]. EGCG prevented neuronal cell death and improved cerebral function after traumatic brain injury in rats [23]. Many studies have also investigated the molecular mechanisms underlying their neuroprotective function in various neurodegenerative diseases [24,25]. Therefore, EGCG might have protective effects against UV irradiation-induced cell death in nervous tissues and cells. However, whether EGCG can protect against UV radiation-induced damage in neuronal cells has yet to be reported. The rat pheochromocytoma cell line PC12, which displays phenotypic characteristics of both adrenal chromaffin cells and sympathetic neurons, is a useful system for exploring neuroprotective drugs [26,27]. Therefore, PC12 cells were used in this study to evaluate the neuroprotective effect of EGCG on UV irradiation-induced apoptosis in PC12 cells and investigate the underlying protective mechanism.

Materials and Methods

1. Cell culture, UV irradiation and EGCG treatment

Rat pheochromocytoma PC12 cells were maintained in RPMI 1640 medium containing 10% horse serum, 5% fetal bovine serum (Gibco BRL, Gaithersburg, MD, USA) inactivated by heat, 100 U mL^{-1} of penicillin, and 100 mg mL^{-1} of streptomycin in a humidified atmosphere containing 5% CO_2 at 37℃. The culture medium was removed and the cells were washed with phosphate-buffered saline (PBS), followed by irradiation with UV using a UVP CL-1000 ultraviolet crosslinker (Transilluminators, Upland, CV, USA). The dose of UV irradiation is indicated in the Figure legend. EGCG was purchased from Sigma-Aldrich (St. Louis, MO, USA) and dissolved with sterile distilled water. The original culture medium was then added back to cells in both treated and control groups.

2. Cell viability based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cell viability was determined by MTT assay, which is based on the reduction of MTT into a visible dark-blue formazan product in viable cells with active mitochondria, thus providing an indirect measure of cell viability [28]. Briefly, PC12 cells were plated into 96-well cell culture plates and exposed to

UV alone or pretreated with different concentrations of EGCG. Next, a final concentration of MTT (0.1 mg/mL) was added to the culture medium at the respective time points and incubated at 37℃ for 4 hours. The reaction product of MTT was dissolved in dimethylsulfoxide. Optical density was then measured at reference wavelengths of 570 nm and 630 nm on an ELx800 UV ELISA reader (BioTek Instruments, Winooski, VT, USA). The background absorbance of medium in the absence of cells was subtracted.

3. Agarose gel electrophoresis for assessment of DNA fragmentation

Oligonucleosomal fragmentation of genomic DNA was assessed using an Apopladder kit (TaKaRa Shuzo, Kyoto, Japan) according to the manufacturer's instructions. Briefly, cells were lysed with 200 μL of cell lysis buffer and centrifuged at 1,100 g for 10 minutes. The supernatant was incubated at 56℃ for 1 hour after adding 20 μL of 10% sodium dodecyl sulfate (SDS) and 20 μL of proteinase K (20 mg/mL). Next, 1 μg of RNase was added to the reaction and incubated at 37℃ for 1 hour. DNA was extracted and precipitated overnight at −20℃ in a precipitant mixture containing 0.95 mL of ethanol and centrifuged at 12,000 g for 15 minutes at 4℃. The DNA pellet was re-suspended in 20 μL of Tris acetate-EDTA buffer. An aliquot of each sample was electrophoresed at 80 V for 2 hours on 1.5% agarose gels containing SYBR™ (safe DNA gel stain, Bionerr, Daejeon, Korea). DNA bands were visualized with a UVP transilluminator/Polaroid camera system (UVP Laboratories, Upland, CA, USA).

4. 4′,6-diamidino-2-phenylindole (DAPI) staining

PC12 cells at a density of 1.0×10^5 cells/mL were plated into 24-well plate overnight and pretreated with 200 mM EGCG for 24 hours. The medium was removed and the cells were washed with PBS solution twice, followed by irradiation with UV at 10 mJ/cm^2 or 20 mJ/cm^2. These cells were further incubated at 37℃ in a humidified atmosphere containing 5% CO_2 for additional 24 hours. After removal of the culture medium, the cells were washed twice with PBS and fixed with ice-cold methanol for 15 minutes at room temperature. The fixed cells were washed again with PBS and stained with 10 μg/mL of DAPI for 10 minutes in the dark. After washing twice with PBS, the cells were examined under a fluorescence microscope (Leica Microsystems, Wetzlar, Germany) to observe the morphology of the nucleus.

5. Detection of ROS production

ROS production was monitored with a fluorescence spectrometer (Hitachi F-4500; Hitachi, Tokyo, Japan) using 2', 7'-dichlorofluorescin diacetate (DCF-DA). Briefly, cells were plated onto a 96-well cell culture plate and treated with UV radiation or EGCG. DCF-DA (25 μM) was then added to the media and cultured at 37℃ for 10 minutes. Emission was measured at a wavelength of 530 nm.

6. Western blot analysis

The PC12 cells were washed twice with PBS, and mixed with a cell lysis buffer (1% NP-40, 500 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM Benzamid, and 1 μL/mL trypsin inhibitor) containing an Xpert Protease Inhibitor Cocktail Solution and an Xpert Phosphatase Inhibitor Cocktail Solution (GenDEPOT, Katy, TX, USA). Cell lysates were incubated at 4℃ for 20 minutes and centrifuged at 12,000 × g for 20 minutes at 4℃. Supernatants were collected and the protein concentrations were determined by BSA protein assay (Thermo Fisher Scientific, Rockford, IL, USA). Protein extracts (50 μg) were boiled for 5 minutes with SDS-sample buffer and then resolved via 8-15% polyacrylamide gel electrophoresis. Proteins were electroblotted onto a nitrocellulose membrane and blocked with 5% skim milk (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) for 1 hour, followed by incubation with anti-poly (ADP-ribose) polymerase (PARP), anti-Bax, anti-Bcl-2, anti-Cyt *c*, anti-cleaved caspase-9, anti-cleaved caspase-3, anti-p-ERK, anti-ERK, anti-p-p38, anti-p38, anti-p-JNK, anti-JNK (Cell Signaling Technology, Danvers, MA, USA) and anti-β-actin antibodies (Santa Cruz Biotechnology, Dallas, TX, USA) for 4 hours at room temperature. Blots were subsequently washed three times in TBS-T for 5 minutes each and incubated with specific horseradish peroxidase (HRP)-conjugated secondary antibodies (Sigma, St. Louis, MO, USA) at room temperature for 1 hour. Bound antibodies were visualized using an Immobilon Western Chemiluminescent HRP Substrate (Millipore, Burlington, MA, USA).

7. Measurement of caspase activity

Caspase activity was assayed using a caspase-3 activity

assay kit (Calbiochem, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, PC12 cells were grown on 100 mm^2 cell culture dishes and irradiated with UV alone or pretreated with EGCG for the indicated time. After removing media from culture dishes, cells were collected, washed with PBS, and resuspended in cell lysis buffer, followed by incubation on ice for 10 minutes. The lysates were centrifuged at 14,000 rpm for 20 minutes. Supernatants were collected and the protein concentrations were determined with BCA assay (Pierce, Rockford, IL, USA). The cell lysate (50 μL) was then mixed with a reaction buffer containing DEVE-*p*NA substrate (200 μM) and incubated at 37℃ for 24 hours. Absorbance was then measured at 405 nm on an ELx800 UV ELISA reader.

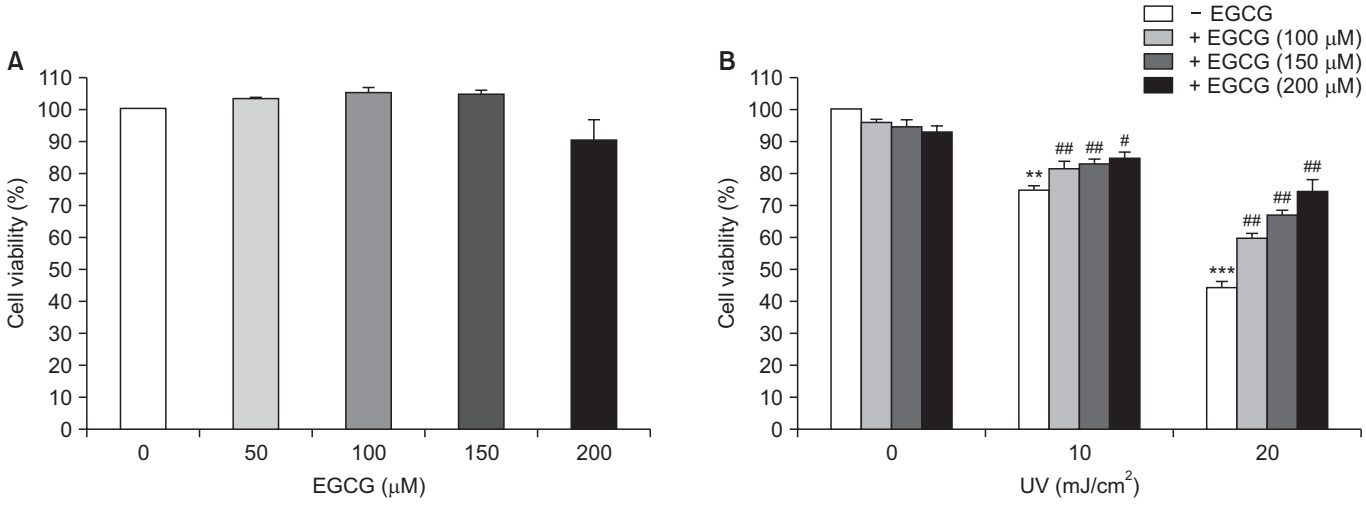

Fig. 1. Effect of (–)-epigallocatechin-3-gallate (EGCG) on the viability of ultraviolet (UV)-irradiated PC12 cells. (A) PC12 cells were treated with different doses of UV irradiation with or without pretreatment with 200 μM EGCG for 24 hours (B) and irradiated with 10 or 20 mJ/cm^2 UV in the presence of 200 μM EGCG for the indicated time. Cell viability was estimated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Results are presented as mean ± standard error of the mean of three independent experiments.
$^{**}p < 0.01$, $^{***}p < 0.001$ compared to the control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to UV-irradiated group.

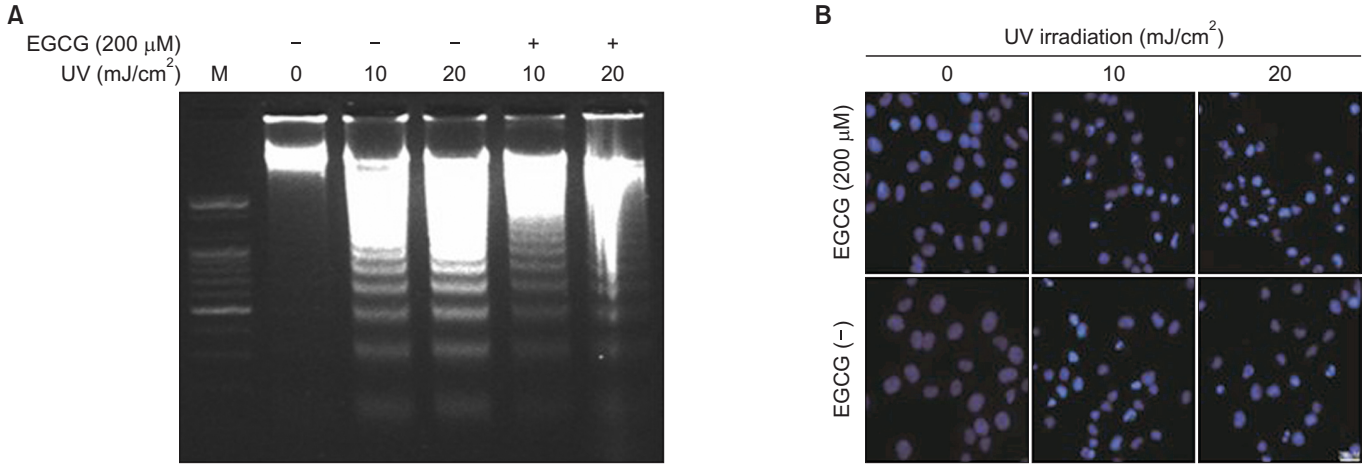

Fig. 2. Effect of (–)-epigallocatechin-3-gallate (EGCG) on ultraviolet (UV) irradiation-induced apoptosis in PC12 cells. (A) DNA was prepared from cells irradiated with UV at 10 or 20 mJ/cm^2 with or without pretreatment with 200 μM EGCG for 24 hours, followed by electrophoresis and visualization with SYBR. (B) Changes in nuclear morphology were observed using 4′,6-diamidino-2-phenylindole (DAPI) staining assay. The cells fixed with ice-cold methanol after treatment were stained with 10 μg/mL of DAPI for 10 minutes in the dark and examined under a florescence microscope after washing twice with phosphate-buffered saline. Results are representative of three independent experiments (×40 magnification).

Results

1. Effects of EGCG on cell viability in UV-irradiated PC12 cells

The MTT assay was used to determine the cytotoxic effects of UV irradiation on PC12 cells with or without EGCG pretreatment (200 μM) at the indicated time course. Cell viability was dramatically decreased after UV irradiation. The survival rate of PC12 cells was about 50% at 24 hours after irradiation at a dose of 100 mJ/cm^2 (Fig. 1A). However, pretreatment with EGCG significantly rescued the decrease in cell viability caused by UV irradiation in a dose-dependent manner (Fig. 1B).

2. EGCG ameliorates apoptosis in UV-irradiated PC12 cells

UV irradiation at 10 or 20 mJ/cm^2 for 24 hours induced DNA fragmentation in PC12 cells, resulting in a DNA ladder pattern, which was suppressed by pretreatment with 200 μM EGCG (Fig. 2A). To further analyze the effect of EGCG on the apoptosis of PC12 cells induced by UV irradiation, changes in nuclear morphology were detected via DAPI staining after UV irradiation at both 10 and 20 mJ/cm^2 with or without EGCG pretreatment (200 μM). As expected, UV exposure increased the number of condensed and fragmented nuclei with enhanced fluorescent intensities in PC12 cells compared with control (untreated). However, pretreatment with EGCG obviously reduced the number of condensed and fragmented nuclei in UV-irradiated PC12 cells (Fig. 2B). This result demonstrated that EGCG ameliorated UV-induced apoptosis of PC12 cells.

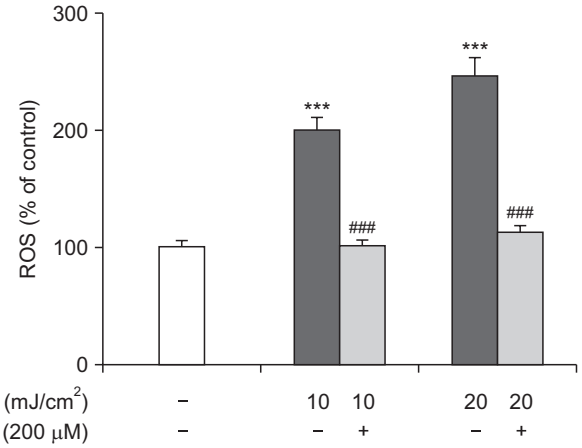

Fig. 3. Effect of (–)-epigallocatechin-3-gallate (EGCG) on reactive oxygen species (ROS) production in ultraviolet (UV)-irradiated PC12 cells. PC12 cells were pretreated with 200 μM EGCG for 24 hours prior to UV irradiation. The culture medium was removed and cells were washed and irradiated with UV at 10 or 20 mJ/cm^2 for 24 hours. Cells were then incubated with 2', 7'-dichlorofluorescin diacetate (DCF-DA) solution for 10 minutes. After washing with 1× Hanks' Balanced Salt Solution (HBSS) buffer, the intracellular levels of ROS were measured as DCF fluorescence. Results are representative of three independent experiments. Data are expressed as mean ± standard error of the mean.
***p < 0.001 compared to the control; $^{###}p$ < 0.001 compared to UV-irradiated group.

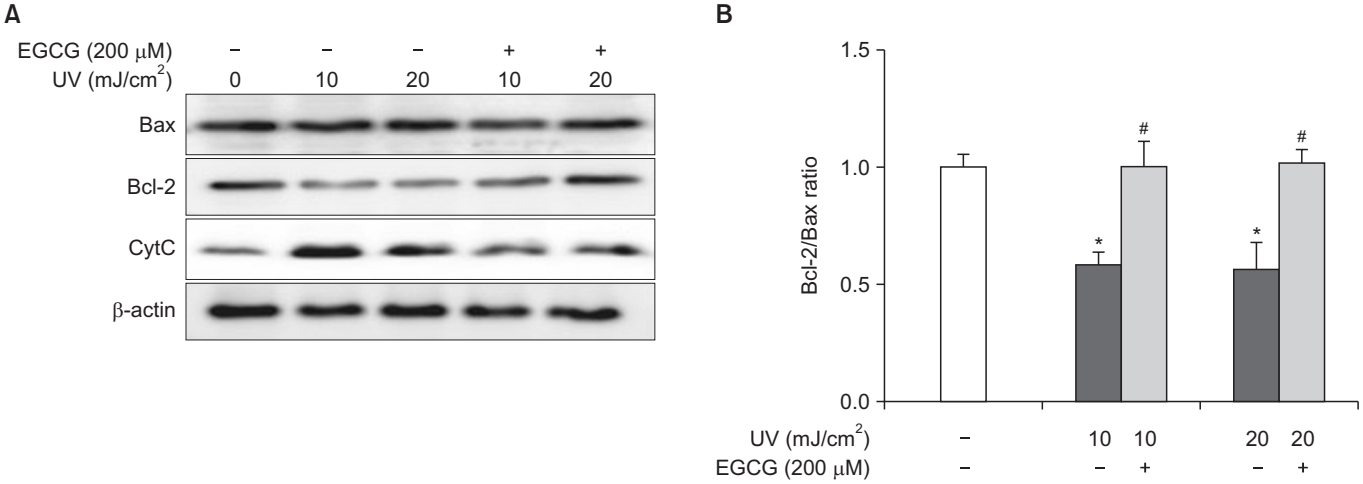

Fig. 4. Effect of (–)-epigallocatechin-3-gallate (EGCG) on the expression of apoptosis-related proteins in ultraviolet (UV)-irradiated PC12 cells. (A) Protein levels of Bcl-2, Bax, and cytochrome *c* (CytC) in PC12 cells irradiated with UV at 10 or 20 mJ/cm^2 for 24 hours, with or without 200 μM EGCG, were determined by western blot. (B) Bcl-2 and Bax expression was measured by densitometry and the relative level of protein expression was normalized to β-actin. Results are representative of three independent experiments. Data are expressed as mean ± standard error of the mean.
*p < 0.05 compared to the control; $^{\#}p$ < 0.05 compared to UV-irradiated group.

3. EGCG reduces ROS production in UV-irradiated PC12 cells

To investigate the scavenging effects of EGCG on ROS production, the ROS levels were measured using DCF-DA in UV-irradiated PC12 cells with or without pretreatment with 200 μM EGCG. UV irradiation at a dose of 10 to 20 mJ/cm^2 enhanced

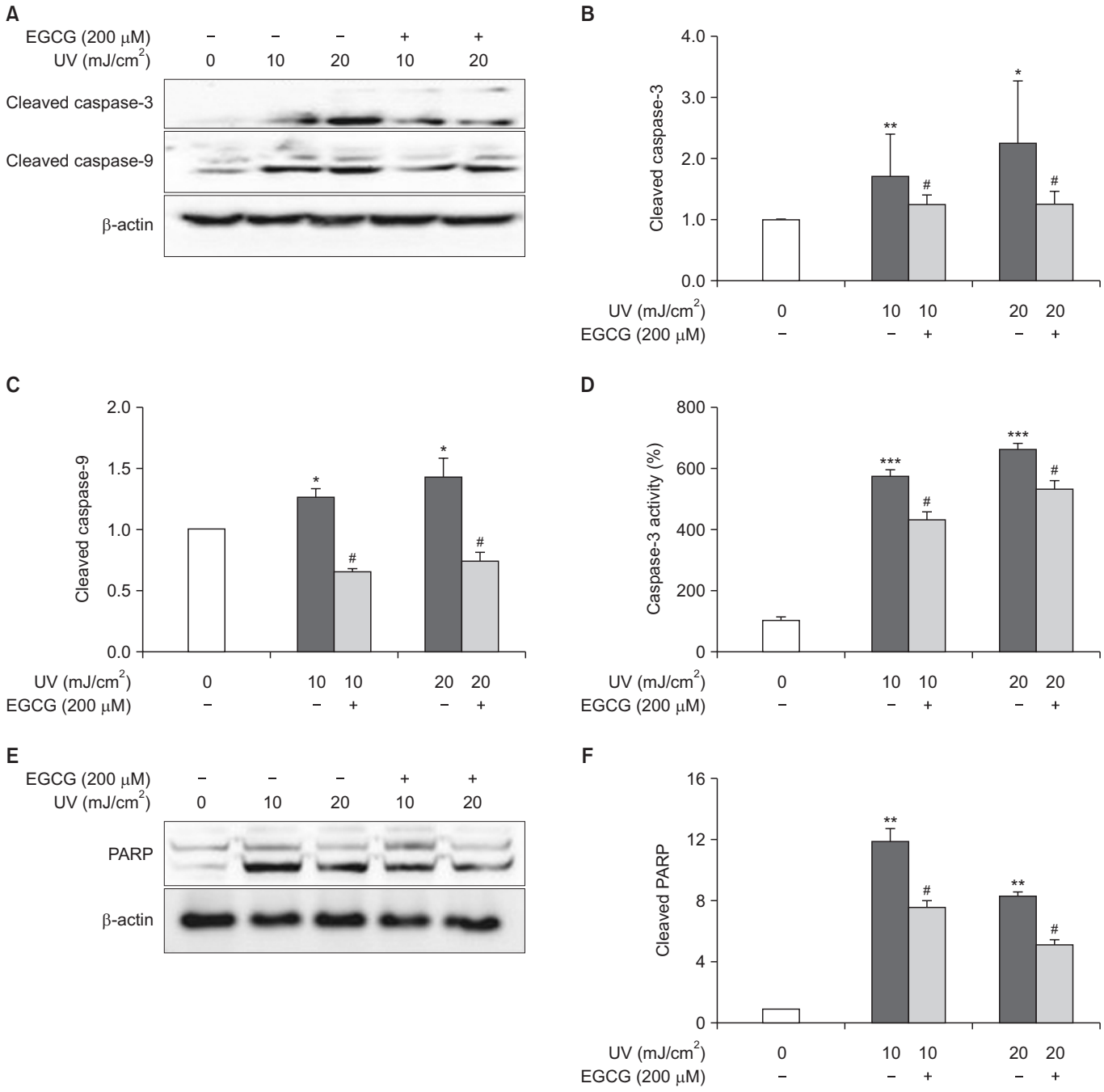

Fig. 5. Effect of (–)-epigallocatechin-3-gallate (EGCG) on caspase activation in ultraviolet (UV)-irradiated PC12 cells. (A-C) Protein levels of cleaved caspase-3 and caspase-9 in PC12 cells irradiated by UV at 10 or 20 mJ/cm^2 with or without 200 μM EGCG pretreatment for 24 hours were measured by western blot. (D) Caspase-3 activity in 10 or 20 mJ/cm^2 UV-irradiated PC12 cells with or without 200 μM EGCG pretreatment for 24 hours. Enzymatic activity of caspase-3 protease was determined by incubating 50 μg of total protein with DEVD-*p*NA substrate (200 μM). (E, F) Protein levels of cleaved poly (ADP-ribose) polymerase (PARP) in 10 or 20 mJ/cm^2 UV-irradiated PC12 cells for 24 hours with or without pretreatment with 200 μM EGCG were measured by Western blot. Results are representative of three independent experiments. Data are expressed as mean ± standard error of the mean.
**p* < 0.05, ***p* < 0.01, ****p* < 0.001 compared to the control; #*p* < 0.05 compared to UV-irradiated group.

the generation of ROS in a dose-dependent manner. In contrast, EGCG significantly inhibited ROS production compared to that of UV-irradiated PC12 cells (Fig. 3). These results suggest that EGCG scavenges the ROS induced by UV irradiation in PC12 cells.

4. EGCG regulates the expression of Bcl-2 family and cytochrome *c* in UV-irradiated PC12 cells

The role of mitochondrial-dependent apoptotic pathways in UV irradiation-induced apoptosis was investigated by analyzing the expression of Bax and Bcl-2 as Bcl-2 family proteins and the cytochrome *c* levels via Western blot analysis. UV irradiation at 10 or 20 mJ/cm^2 for 24 hours significantly reduced the expression of Bcl-2, an inhibitor of cytochrome *c* released from mitochondria into cytoplasm. The expression of Bax and cytochrome *c* was upregulated by UV irradiation (10 or 20 mJ/cm^2). However, pretreatment with 200 μM EGCG increased the expression of Bcl-2. It also ameliorated the upregulation of Bax and cytochrome *c* induced by UV (Fig. 4A). The Bax-to-Bcl-2 ratio is a significant parameter determining apoptosis. A high ratio denotes a low apoptotic threshold for cytochrome *c* release from mitochondria to cytosol, whereas a low ratio indicates a high apoptotic threshold [29]. The Bcl-2:Bax ratio was decreased about 50% by UV irradiation. However, pretreatment with EGCG significantly restored the Bcl-2/Bax ratio similar to that of the control group (Fig. 4B).

5. EGCG inhibits the activation of caspase-3 and caspase-9 in UV-irradiated PC12 cells

Caspases play an essential role in inducing and regulating apoptosis [30]. The decreased Bcl-2/Bax ratio and subsequent release of cytochrome *c* into cytosol usually result in the activation of caspases, including initiator caspase-8 and caspase-9 and their downstream effector caspase-3 [31]. Therefore, it was further examined whether the activation of caspases played a role in the apoptosis of PC 12 cells induced by UV irradiation. As shown in Fig. 5A-C, the expression of both caspase-3 and caspase-9 was significantly increased by UV at 10 or 20 mJ/cm^2 compared with the control group. However, it was obviously reduced after pretreatment with 200 μM EGCG. In addition, the caspase-3 activity that was increased in PC12 cells irradiated by UV at 10 or 20 mJ/cm^2 for 24 hours was blocked by pretreatment with 200 μM EGCG (Fig. 5D). Activation of caspase-3 is monitored by effective cleavage of PARP as its substrate [32]. The cleavage of PARP was detected by western blot in UV-irradiated PC12 cells pretreated with 200 μM EGCG. As shown in Fig. 5E and 5F, the concentration of cleaved PARP was increased by UV irradiation. Such increase was blocked by pretreatment with 200 μM EGCG. However, the expression of caspase-8 in UV-irradiated PC12 cells was not affected by EGCG pretreatment (data not shown).

6. EGCG ameliorates UV-induced apoptosis by inhibiting phosphorylation of p38 in PC12 cells

The mitogen-activated protein kinase (MAPK) pathways play an important role in regulating apoptosis [33]. The role of MAPK pathway, including ERK, p38, and JNK in UV-induced apoptosis of PC12 cells was assessed. UV irradiation significantly increased the phosphorylation of p38 and JNK, but decreased that of ERK (Fig. 6). In addition, whether the inhibitory effect of EGCGs on apoptosis was associated with changes in MAPKs was investigated. Cells were incubated with 200 μM EGCG for 1 hour, exposed to UV 10 or 20 mJ/cm^2 irradiation, and harvested 24 hours later. The UV-induced phosphorylation of p38 and p-JNK was suppressed by EGCG pretreatment, whereas UV-downregulated phosphorylation of ERK was rescued (Fig. 6). These findings suggest that EGCG ameliorated UV-induced apoptosis via JNK inactivation and ERK activation in PC12 cells.

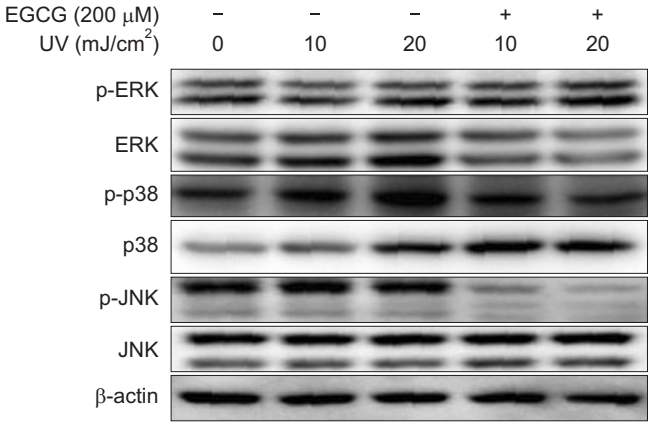

Fig. 6. Effect of (−)-epigallocatechin-3-gallate (EGCG) on mitogen-activated protein kinase (MAPK) activation in ultraviolet (UV)-irradiated PC12 cells. Phosphorylated MAPK (p-ERK, p-p38, and p-JNK) in PC12 cells irradiated by UV at 10 or 20 mJ/cm^2 with or without 200 μM EGCG pretreatment for 24 hours were measured by Western blot analysis.

Discussion

UV irradiation induces a variety of biological effects, including the induction of programmed cell death. It not only causes DNA damage by generating ROS, but also induces nonspecific oxidative damage known to result in mitochondrial impairment and activation of death receptors [9]. Activation of caspase, calpain, JNK, and mitochondrial apoptosis is involved in neuronal cell death induced by UV irradiation [7,10,11]. In the present study, UV irradiation resulted in DNA fragmentation, augmented ROS production, increased the expression of cleaved caspase-3 and caspase-9, enhanced caspase-3 activity, and cleaved PARP. In addition, the Bcl-2/Bax ratio and cytochrome *c* release into cytosol were increased in UV-irradiated PC12 cells, while such increases were blocked by pretreatment with EGCG. Increased ROS production in the mitochondria is known to induce formation of mitochondrial permeability transition pore that perturbs mitochondrial structure and function, resulting in the release of cytochrome *c*, which subsequently induces apoptosis [12]. Taken together, these results suggest that UV irradiation induces apoptosis of PC12 cells by generating oxidative stress via caspase-dependent mitochondrial pathway, consistent with previous studies involving neuronal cells [9,10,34].

EGCG is a major catechin in green tea that has neuroprotective and neurorestorative effects [35,36]. Our group has previously reported that EGCG rescued lipopolysaccharide-impaired adult hippocampal neurogenesis by suppressing toll-like receptor 4-nuclear factor-κB signaling pathway in mice [37]. In the present study, EGCG rescued cell viability decreased by UV irradiation and blocked DNA fragmentation and chromosome condensation, indicating that EGCG prevents UV-induced apoptosis in PC12 cells.

It has been demonstrated that UV irradiation strongly stimulates the synthesis of ROS such as superoxide anion radical, hydroxyl radical, hydrogen peroxide, and peroxynitrite, which are highly reactive and destructive oxidants [38]. EGCG is a potent antioxidant. Its antioxidant effects have been extensively studied in diverse cells [39-41]. A recent study has reported that the antioxidant and free-radical scavenging effects of EGCG are responsible for its neuroprotective effects in rats and cytotoxicity in PC12 cells [42]. In the present study, UV irradiation dose-dependently increased the ROS generation in PC12 cells. However, treatment with EGCG markedly reduced ROS production induced by UV irradiation, suggesting that EGCG has an antioxidant effect in UV-induced neuronal cells. This finding also demonstrates that UV irradiation-induced apoptosis might be driven by oxidative stress, while the ROS-scavenging effects of EGCG might play an important role in protection against cell death induced by UV irradiation. Our results are consistent with a previous study reporting the antioxidant effects of EGCG mediated via suppression of 1-methy-4-phenyl-pyridine-induced oxidative stress in PC12 cells [43].

Anti-apoptotic proteins (e.g., Bcl-2 and Bcl-xL) and pro-apoptotic proteins (e.g., Bax and Bid) belonging to Bcl-2 family are important modulators of cell death [29]. The altered anti-apoptotic-to-pro-apoptotic ratio of Bcl-2 proteins was significant in determining the occurrence of apoptosis [44]. In the present study, the Bcl-2-to-Bax ratio was increased significantly by EGCG pretreatment compared with that of UV irradiation. It has been reported that EGCG directly regulated the expression of Bcl-2 family proteins such as Bad, Bax, Mdm2, Bcl-2, and Bcl-xL, and Bcl-2 family plays an important role in regulating the release of cytochrome *c* [44]. cytochrome *c* release, a hallmark of apoptosis, is an early event in UV-induced cell death [45]. Our data showed that UV irradiation increased the level of cytochrome *c* release in PC12 cells. Such increase was attenuated by EGCG pre-treatment, suggesting that EGCG modulated the expression of Bcl-2 family proteins and blocked the downstream effectors of mitochondrial apoptotic pathway.

Caspases transduce apoptotic signal cascade. Initiator caspases cleave and activate effector caspases, which then degrade other cellular targets, leading to cell death [15]. The cytochrome *c* released into the cytosol during apoptosis binds to Apaf-1, which then recruits and activates pro-caspase-9 or caspase-8 as initiator caspases, leading to the activation of caspase-3 as one of the effector caspases in apoptotic cells [46]. Activated caspase-3 also plays a central role in the execution of the apoptotic program resulting in the cleavage of PARP during cell death [46]. In the present study, the induction of apoptosis by UV irradiation was accompanied by an increase in caspase-3 activity. However, these effects were attenuated by EGCG pretreatment. In addition, the cleavage of PARP as a later event in apoptosis was increased by UV irradiation, while such increase was blocked by EGCG. These results demonstrate that EGCG inhibits UV irradiation-induced apoptosis by suppressing the activation of caspase-3, which in turn suppresses the cleavage of PARP in PC12 cells. Taken together, our results suggest that EGCG protects PC12 cells against UV irradiation-induced apoptosis by scavenging ROS, regulating the expression of Bcl-2 family proteins, and inhibit-

ing mitochondrial pathway-mediated cytochrome *c* release. The activation of caspase-9 and caspase-3 plays a crucial role in UV irradiation-induced apoptosis of PC12 cells. Thus, EGCG and its derivatives represent potential as neuroprotective drugs for treating neurotoxicity, neural damage, and neurodegenerative diseases.

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Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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