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Protective Effect of (-)-Epigallocatechin Gallate on Nitric Oxide-Induced Apoptosis in Alveolar Epithelial Cells

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The lung is exposed to various stimulants, such as reactive nitrogen species. Alveolar epithelial cells are sensitive to nitric oxide (NO), which is a free radical inorganic gas synthesized from L-arginine by nitric oxide synthase (NOS). NO mediates many biological processes, but excessive NO exposure induces apoptosis in various cell types. (-)-Epigallocatechin-3-gallate (EGCG) has recently been shown to modulate apoptotic pathways. EGCG is the most abundant and most active ingredient of green tea. EGCG has been shown to have anti-carcinogenic, anti-oxidant, anti-inflammatory, neuroprotective, and anti-apoptotic effects. In the present study, we investigated whether EGCG exhibits a protective effect against apoptosis induced by the NO donor sodium nitroprusside (SNP) in human lung epithelial cells. To confirm the anti-apoptotic properties of EGCG, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, 4,6-diamidino-2-phenylindole (DAPI) staining, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay, DNA fragmentation assay, and Western blotting were performed using human alveolar type II L-132 cells. The present results show that SNP induced apoptotic morphological changes, increased the expression levels of the pro-apoptotic protein Bax and enhanced the enzymatic activity of caspase-3 in L-132 human lung epithelial cells. However, EGCG treatment remarkably increased the expression of the anti-apoptotic protein Bcl-2, decreased the expression of Bax, and suppressed the enzymatic activity of caspase-3. The results of the present study revealed that EGCG exerts a protective effect against SNP-induced apoptosis in L-132 human lung epithelial cells. (**Korean J Str Res 2011;19:69～77**)

Key Words: (-)-Epigallocatechin-3-gallate, Sodium nitroprusside, Nitric oxide, Human lung epithelial cells, Apoptosis

INTRODUCTION

The lung is composed of many different types of cells, including endothelial cells, epithelial cells, fibroblasts, and inflammatory cells. Alveolar epithelium lines the alveolar air sacs, which are involved in gaseous exchange. The alveolar epithelium is predominantly comprised of two specialized epithelial cell types: squamous alveolar epithelial type I cells, which constitute approximately 93% of the alveolar epithelial surface area, and cuboidal alveolar epithelial type II cells, which comprise the remaining 7% of the alveolar epithelial surface area and 67% of epithelial cells. The alveolar type II cells are regarded as the "defender of the

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Received February 15, 2011, Revised March 10, 2011
Accepted March 12, 2011

alveolus" (Fehrenbach, 2001). Alveolar type II cells contribute to the following four major functions: (i) synthesis and secretion ofsurfactant, (ii) xenobiotic metabolism, (iii) transepithelial movement of water, and (iv) regeneration of the alveolar epithelium following lung injury. Therefore, alveolar type II cells play important roles in normal pulmonary activity and in responses of the lungs to toxic compounds, which may cause lung damage (Castranova *et al.,* 1988). Alveolar epithelium is sensitive to oxidative stresses derived from reactive oxygen species and nitrogen species, such as nitric oxide (NO) and peroxynitrite ($ONOO^-$) (Freeman *et al.,* 1993).

NO is a free radical inorganic gas synthesized from L-arginine by three isoforms of nitric oxide synthase (NOS). NO mediates many biological functions, including vasodilation, inhibition of platelet aggregation, neurotransmission, and immune reactions. However, excessive NO exposure induces apoptosis in various types of cells, such as macrophages and megakaryocytes (Battinelli *et al.,* 2000).

Apoptosis (programmed cell death) is a form of cell death that occurs during several pathological situations in multi-cellular organisms and contributes to cell replacement, tissue remodeling, and removal of damaged cells under normal conditions (DeLong, 1998). Numerous factors are involved in the regulation of apoptosis. In particular, the members of the Bcl-2 family of proteins play pivotal roles in the regulation of apoptosis, and they activate caspases that mediate the cleavage of apoptosis regulators. Caspases, a family of cysteine proteases, are integral parts of the apoptotic pathway. The Bcl-2 family proteins are classified as either anti-apoptotic proteins or pro-apoptotic proteins based on their function. The balance between pro-apoptotic and anti-apoptotic Bcl-2 family members determines the mitochondrial response to apoptotic stimuli (Upadhyay *et al.,* 2003).

Green tea belongs to the Theaceae family and is derived from two main varieties: Camellia sinensis var. sinensis and Camellia sinensis var. assamica (Graham, 1992; Sang S *et al.,* 2003). (-) Epigallocatechin gallate (EGCG) is a constituent of green tea that has been reported to have anti-inflammatory, neuroprotective, anti-tumor, and anti-oxidant effects as a free radical scavenger. It was recently reported that EGCG modulates apoptotic pathways (Kelly *et al.,* 2001; Bastianetto *et al.,* 2006; Sutherland *et al.,* 2006; Yang *et al.,* 2006; Syed *et al.,* 2007).

In the present study, we investigated the protective effect of EGCG against NO-induced apoptosis in L-132 human alveolar epithelial cells by using sodium nitroprusside (SNP), an NO donor. In this study, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay, 4,6-diamidino-2-phenylindole (DAPI) staining, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay, DNA fragmentation assay, Western blot analysis, and caspase-3 enzyme activity assay were performed.

MATERIALS AND METHODS

1. Drugs and reagents

EGCG and SNP were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The MTT assay kit was purchased from Boehringer Mannheim GmbH (Mannheim Germany). The DNA fragmentation assay kit was obtained from TaKaRa (Shiga, Japan), and the caspase-3 assay kit was purchased from CLONTECH (Palo Alto, CA, USA).

2. Cell culture

Cells derived from human alveolar type II cells, L-132 cells, were purchased from the Korean Cell Line Bank (KCLB; Seoul, Korea). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL) at 37°C in 5% CO_2, 95% O_2 in a humidified cell incubator. The medium was changed every 2 days. The cells were plated onto culture dishes at a density of 2×10^4 cells/cm^2 12 h prior to treatment with EGCG. Cells from passages 8 to 25 were used in the experiments.

3. MTT cytotoxicity assay

Cell viability was determined using the MTT assay kit according to the manufacturer's protocol. To determine the cytotoxicity of SNP, the cells were treated with SNP at concentrations of 100 μM, 500 μM, 1 mM, 2 mM, and 4 mM for 12 h. To analyze the protective effect of EGCG against SNP-induced cell death, cells were pre-treated with EGCG at concentrations of 12.5 μM/ml, 25 μM/ml, 50 μM/ml, 100 μM/ml, and 200 μM/ml for 1 h prior to SNP treatment. The cells in the control group

were left untreated. Ten microliters of MTT labeling reagent containing 5 mg/ml MTT in phosphate-buffered saline (PBS) was added to each well, and the plates were incubated for 4 h. Each well was added with 100 μl of a solubilization solution containing 10% sodium dodecyl sulfate (SDS) in 0.01 M hydrochloric acid (HCl), and the cells were incubated for another 12 h. The absorbance was then measured with a microtiter plate reader (Bio-Tek, Winooski, VT, USA) at a test wavelength of 595 nm with a reference wavelength of 690 nm. The optical density (O.D.) was calculated as the difference between the absorbance at the reference wavelength and that observed at the test wavelength. Percent viability was calculated as follows: (O.D. of drug-treated sample/control O.D.) ×100.

4. TUNEL assay

For in situ detection of apoptotic cells, TUNEL assay was performed using the ApoTag® peroxidase in situ apoptosis detection kit. Cells were cultured on 4-chamber slides (Nalge Nunc International, Naperville, IL, USA) at a density of 2×10^4 cells/chamber. The cells were pre-treated with 100 μM/ml EGCG for 1 h prior to 2 mM SNP treatment and incubated for another 12 h. After treatment with EGCG and SNP, the cells were washed with phosphate buffered saline (PBS) and fixed by incubation in 4% paraformaldehyde (PFA) for 10 min at 4°C. The fixed cells were then incubated with digoxigenin-conjugated dUTP in a terminal deoxynucleotidyl transferase (TdT)-catalyzed reaction for 60 min at 37°C in a humidified atmosphere and immersed in stop/wash buffer for 10 min at room temperature. The cells were then incubated with anti-digoxigenin antibody conjugated with peroxidase for 30 min. DNA fragments were stained using 3,3-diaminobenzidine (DAB; Sigma Chemical Co.) as the substrate for peroxidase.

5. DAPI staining

For DAPI staining, the cells were cultured on 4-chamber slides (Nalge Nunc International). The cells were pre-treated with 100 μM/ml EGCG for 1 h prior to 2 mM SNP treatment and incubated for another 12 h. After treatment with EGCG and SNP, the cells were fixed by incubation in 4% PFA for 30 min. The cells were washed in PBS and then incubated in 1 μg/ml DAPI solution (Sigma Chemical Co.) for 30 min in the dark. The cells were then observed under a fluorescence microscope (Zeiss, Oberköchen, Germany).

6. DNA fragmentation

For detection of apoptotic DNA cleavage, a DNA fragmentation assay was performed using the ApopLadder EX™ DNA fragmentation assay kit. The cells were treated with EGCG and SNP and then lysed with 100 μl of lysis buffer. The lysate was incubated with 10 μl of 10% sodium dodecyl sulfate (SDS) solution containing 10 μl of Enzyme A at 56°C for 1 h followed by treatment with 10 μl of Enzyme B at 37°C for 1 h. After adding 70 μl of precipitant and resuspending the resultant pellet in Tris-EDTA (TE) buffer, genomic DNA was visualized by electrophoresis in a 2% agarose gel containing ethidium bromide.

7. Western blot analysis

The cells were treated with EGCG and SNP and incubated for 12 h. After treatment with EGCG and SNP, the cells were collected by trypsinization and centrifugation. The supernatant was removed, and the cell pellets were lysed in a lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.5% deoxycholic acid, 1% Nonidet P40, 0.1% SDS, 1 mM PMSF, and 100 mg/ml leupeptin. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad). Thirty micrograms of protein was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane. Mouse Bax antibody (1 : 1,000; Santa Cruz Biotech, Santa Cruz, CA, USA) and mouse Bcl-2 antibody (1 : 1,000; Santa Cruz Biotech) were used as primary antibodies. Horseradish peroxidase-conjugated anti-mouse antibody for Bax and Bcl-2 (1 : 2,000; Amersham Pharmacia Biotech GmbH, Freiburg, Germany) were used as secondary antibodies. Band detection was performed using the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech GmbH).

8. Caspase-3 enzyme activity assay

The enzymatic activity of caspase-3 was measured using the ApoAlert® caspase-3 assay kit according to the manufacturer's protocol. The colorimetric assay is based on spectrophotometric detection of the chromophore *p*-nitroaniline (*p*NA) after cleavage from the labeled caspase-specific substrates by caspases. The

caspase-3-specific substrate used in the present study was DEVD-*p*NA, and the rate of DEVD-*p*NA cleavage was measured in order to assay the caspase-3 enzyme activity. In brief, after treatment with EGCG and SNP, the cells were lysed with 50 μl of chilled cell lysis buffer. Fifty microliters of 2× reaction buffer (containing DTT) and 5 μl of the appropriate conjugated substrate (DEVD-*p*NA) at a concentration of 1 mM were added to each lysate. The mixture was incubated in a water bath at 37°C for 1 h, and the absorbance was measured with a microtiter plate reader at a test wavelength of 405 nm. The caspase-3 inhibitor (DEVD-fmk) was used as negative control.

9. Statistical analysis

The results were expressed as the mean±standard error of the mean (SEM). The data were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's *post-hoc* test using WIN SPSS 12.0. Differences were considered statistically significant at $p < 0.05$.

RESULTS

1. Effect of EGCG on viability of SNP-treated L-132 cells

As shown in Fig. 1, the viabilities of cells incubated with SNP at concentrations of 100 μM, 500 μM, 1 mM, 2 mM, and 4 mM for 12 h were 98.83±3.93%, 90.53±2.44%, 83.88±3.82%, 59.18±3.13%, and 25.43±0.61% of the control value, respectively. It was observed that the viability of cells decreased as the concentration of SNP increased. The concentration of SNP was set at 2 mM for the next experiments.

As shown in Fig. 2, the viability of cells exposed to 2 mM SNP for 12 h was 63.84±2.21% of the control value, while the viabilities of the cells pre-treated with EGCG at concentrations of 12.5 μM, 25 μM, 50 μM, 100 μM, and 200 μM for 1 h before exposure to 2 mM SNP increased significantly to 67.18±5.33%, 72.88±7.05%, 80.42±6.51%, 89.32±7.29% and 85.45±9.80%, respectively. MTT assay showed that SNP treatment significantly decreased the viability of cells, while pre-treatment with EGCG exerted a protective effect against SNP-induced cytotoxicity. The concentrations of the EGCG were set at 50 μM and/or 100 μM for the next experiments.

2. Morphological changes induced by SNP and EGCG

The cells were treated with SNP to further confirm the induction of apoptosis by SNP and the protective effect of EGCG in L-132 cells, and the protective effect of EGCG was analyzed

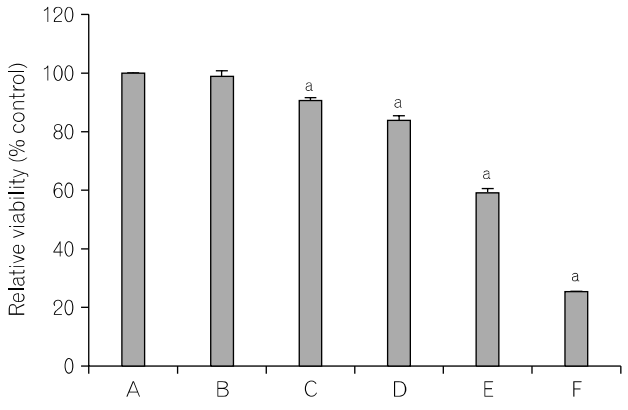

Fig. 1. Sodium nitroprusside (SNP)-induced cytotoxicity. Human lung alveolar epithelial cell line L-132 was incubated with SNP at various concentrations for 12 h prior to the determination of cellular viability using the MTT assay. (A) Control group, (B) 100 μM SNP-treated group, (C) 500 μM SNP-treated group, (D) 1 mM SNP-treated group, (E) 2 mM SNP-treated group, (F) 4 mM SNP-treated group. [a]Represents $p < 0.05$ compared to the control group.

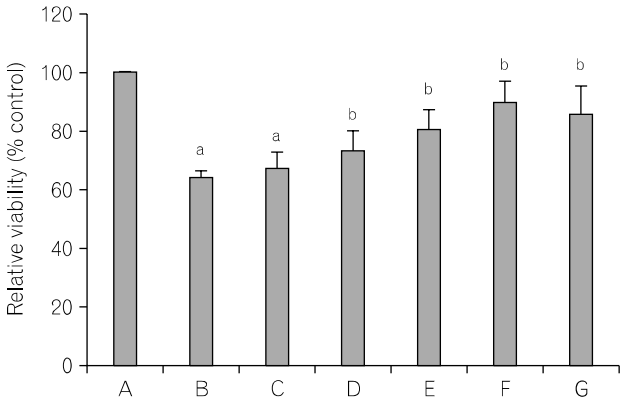

Fig. 2. Protective effect of (-) epigallocatechin gallate (EGCG) against sodium nitroprusside (SNP)-induced cytotoxicity. (A) Control group, (B) 2 mM SNP-treated group, (C) 12.5 μM EGCG-pre-treated and 2 mM SNP-treated group, (D) 25 μM EGCG-pre-treated and 2 mM SNP-treated group, (E) 50 μM EGCG-pre-treated and 2 mM SNP-treated group, (F) 100 μM EGCG-pre-treated and 2 mM SNP-treated group, (G) 200 μM EGCG-pre-treated and 2 mM SNP-treated group. [a]Represents $p < 0.05$ compared to the control group. [b]Represents $p < 0.05$ compared to the SNP-treated group.

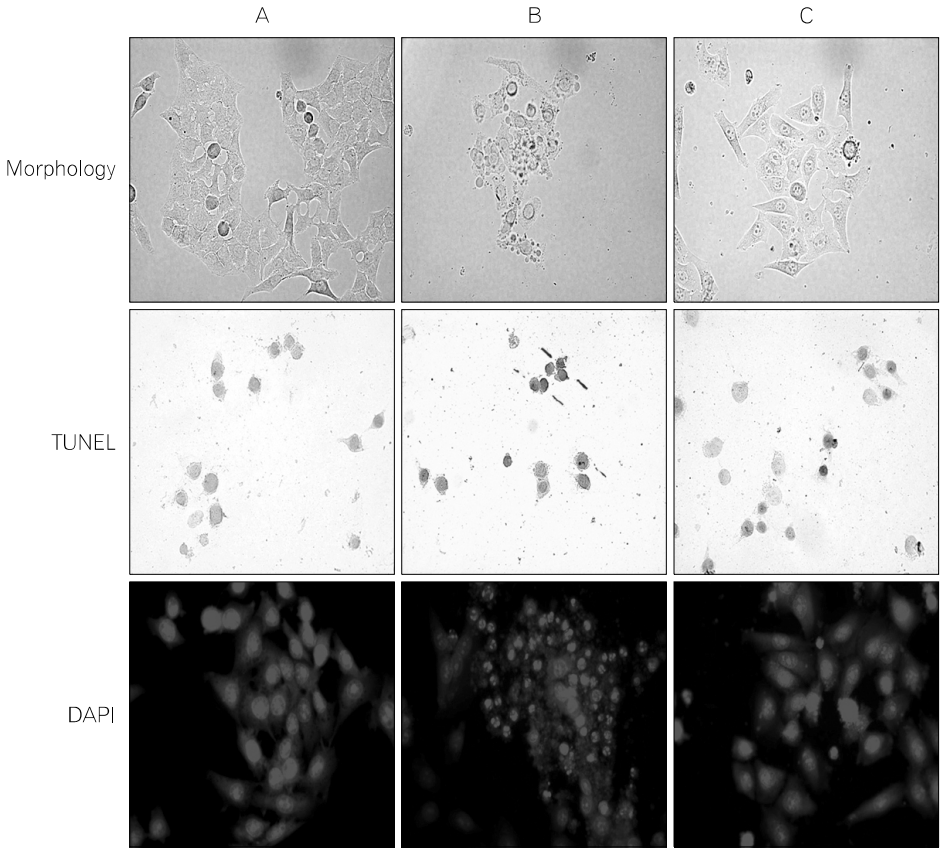

Fig. 3. Morphological observations of cells treated with sodium nitroprusside (SNP) and (-) epigallocatechin gallate (EGCG). (A) Control group, (B) 2 mM SNP-treated group, (C) 100 μM EGCG-pre-treated and 2 mM SNP-treated group.

using a TUNEL assay, DAPI staining and PI staining. DNA strand breaks occur during apoptosis, and it is known that nicks in DNA molecules can be detected via TUNEL assay. As shown in Fig. 3, TUNEL-positive cells were stained dark brown under the light microscope, and nuclear condensations were observed in the cells treated with 2 mM SNP, while the appearance of cells pre-treated with 100 μM EGCG prior to SNP exposure was similar to that of the control cells.

DAPI staining revealed nuclear condensation, DNA fragmentation, and perinuclear apoptotic bodies. Apoptotic bodies, one of the stringent morphological criteria for apoptosis, were remarkably presented in the 2 mM SNP-treated cells, whereas attenuated morphological changes were shown in the cells pre-treated with 100 μM EGCG prior to SNP exposure.

3. Characterization of apoptosis via examination of DNA fragmentation

DNA fragmentation, which reflects the endonuclease activity characteristic of apoptosis, was assessed in order to investigate the

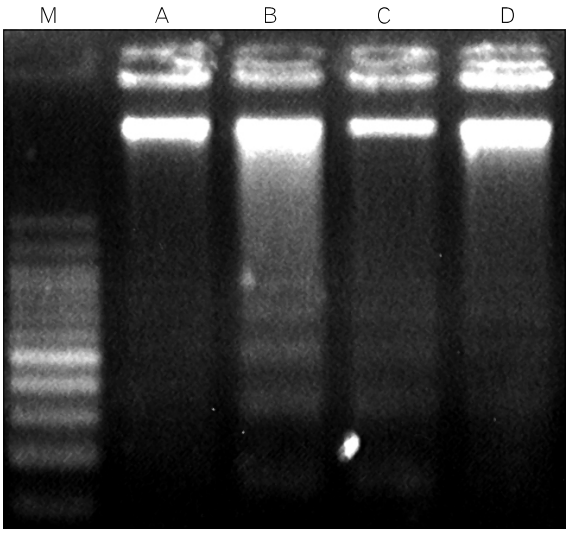

Fig. 4. Electrophoretic examination of the genomic DNA of L-132 human lung epithelial cells. (M) Marker, (A) control group, (B) 2 mM SNP-treated group, (C) 50 μM EGCG-pre-treated and 2 mM SNP-treated group, (D) 100 μM EGCG-pre-treated and 2 mM SNP-treated group.

protective effect of EGCG against SNP-induced cell death. As shown in Fig. 4, treatment with SNP at a concentration of 2 mM for 12 h resulted in the formation of definite fragments, which could be seen via electrophoresis as a characteristic ladder pattern: pre-treatment with 100 μM EGCG for 1 h resulted in a decrease in the intensity of SNP-induced DNA laddering.

4. Western blot analysis of Bcl-2 and Bax protein expression

Increased expression of the Bax protein (26 kDa) and decreased expression of the Bcl-2 protein (25 kDa) were observed in the cells treated with 2 mM SNP. However, the cells pre-treated with 100 μM EGCG showed a decrease in Bax protein expression and an increase in Bcl-2 protein expression (Fig. 5).

5. Caspase-3 enzyme activity analysis

Caspase-3 enzyme activity was measured using DEVD-*p*NA. The amount of DEVD-pNA significantly increased after 12 h of exposure to 2 mM SNP from 4.83±0.09 pmol (the control value) to 8.16±0.02 pmol, whereas pre-treatment with EGCG at concentrations of 50 μM and 100 μM significantly reduced caspase-3 enzyme activity to 5.24±0.02 pmol and 5.12±0.02 pmol, respectively (Fig. 6). In the present results, EGCG inhibited the caspase-3 enzyme activity induced by SNP.

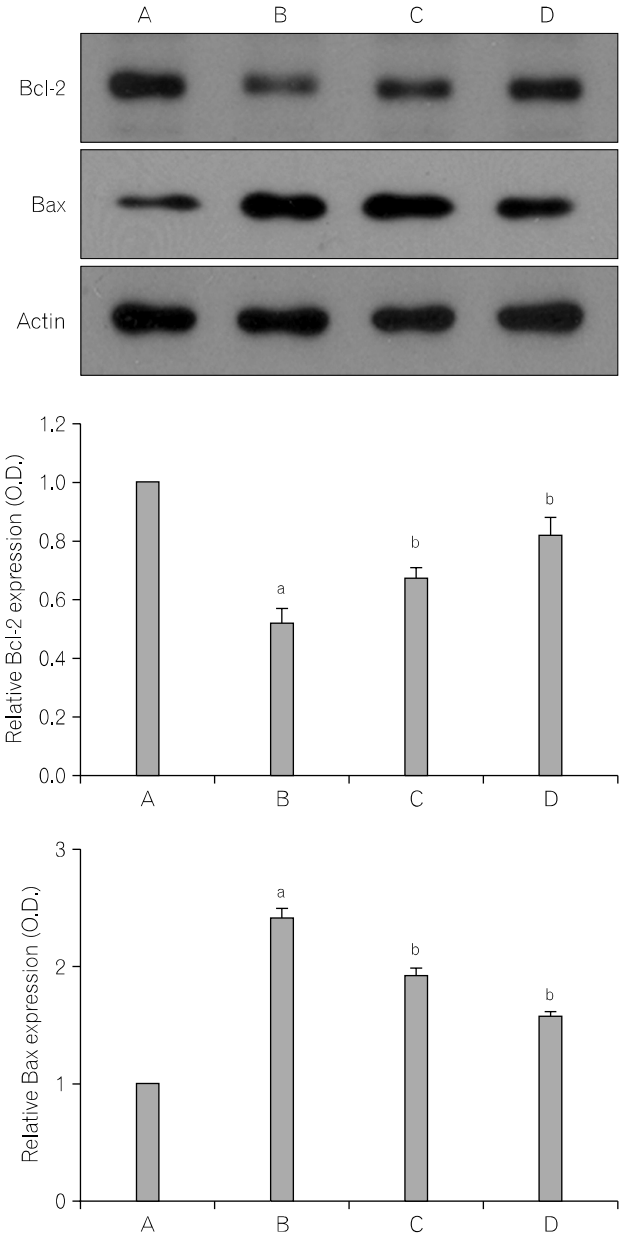

Fig. 5. Results of Western blot analysis of Bax and Bcl-2 protein levels. (A) Control group, (B) 2 mM sodium nitroprusside (SNP)-treated group, (C) 50 μM (-) epigallocatechin gallate (EGCG)-pre-treated and 2 mM SNP-treated group, (D) 100 μM EGCG-pre-treated and 2 mM SNP-treated group.

DISCUSSION

Lung cells are exposed to a variety of free radical species and pollutants. Alveolar epithelial cells are especially sensitive to NO

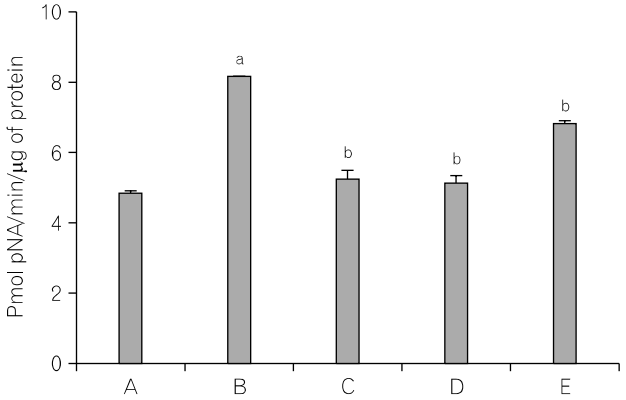

Fig. 6. Inhibitory effect of EGCG on sodium nitroprusside (SNP)-induced caspase-3 enzyme activity. A, Control group; B, 2 mM sodium nitroprusside (SNP)-treated group; C, 50 μM (-) epigallocatechin gallate (EGCG)-pre-treated and 2 mM SNP-treated group, (D) 100 μM EGCG-pre-treated and 2 mM SNP-treated group, (E) DEVD-fmk added SNP-treated group. [a]Represents $p < 0.05$ compared to the control group. [b]Represents $p < 0.05$ compared to the SNP-treated group.

and $ONOO^-$ (Freeman *et al.,* 1993). It was previously reported that NO injured the alveolar epithelial cells and finally led to apoptosis (Yvonne *et al.,* 1997; Lang *et al.,* 2000). NO cytotoxicity has been regarded as a two-edged sword. NO kills invading pathogens or tumor cells and can therefore be regarded as an essential component of the non-specific immune defense system. Actually, NO protects the mitochondria in the anterior pituitary cells and human gingival fibroblasts (Poliandri *et al.,* 2005; Argentin *et al.,* 2006). Therefore, an appropriate level of NO is necessary for cell survival. On the other hand, excessive NO production induces severe symptoms (Messmer *et al.,* 1996; Rachek *et al.,* 2006), and too much NO increases cytotoxicity and results in apoptosis in various cell types, including lymphoblastoids, fibroblast cells, and vascular smooth muscle cells (McLaughlin *et al.,* 2005; Pilane *et al.,* 2005). In general, NO is implicated in a variety of biological signaling and physiological functions. However, NO induces apoptosis by modulating the mitochondrial permeability transition, a process that is linked to the release of apoptogenic factors such as cytochrome *c*. The release of cytochrome *c* is an important step in the activation of specific subgroups of caspases and thus promotes apoptosis. Moreover, NO inhibits the rate-limiting enzyme in DNA synthesis and ribonucleotide reductase and increases the expression of p53. Due to the over-expression of the cell death gene, p53, the ratio of pro-apoptotic Bax to anti-apoptotic Bcl-x_L is increased, leading to NO-induced apoptosis (Kwon *et al.,* 1991; Messmer *et al.,* 1996; Li *et al.,* 1997; Brockhaus *et al.,* 1998; Brookes *et al.,* 2000). In the present results, excessive NO production by SNP induced apoptosis in human lung epithelial cells, whereas EGCG exerted a protective effect against SNP-induced apoptosis.

Apoptosis is a form of regulated cell death in which activation of specific intracellular serine rich proteases (caspases) leads to DNA cleavage and results in cell death. Characteristically, apoptosis accompanies morphological changes such as cell shrinkage, chromatin condensation, and DNA fragmentation (Kerr *et al.,* 1972). In the present study, such apoptosis-induced morphological changes were observed via TUNEL, DAPI, PI and DNA fragmentation assay.

Apoptosis is controlled, in part, by a complex interplay between regulatory proteins. The Bcl-2 family is a group of closely related proteins that plays a major role in apoptosis. They regulate apoptosis in a rheostatic manner. For instance, under conditions of excess Bax, Bax homodimers predominate, leading to apoptosis. Conversely, under conditions of excess Bcl-2, Bcl-2/Bax heterodimers are formed, which leads to inhibition of apoptosis (White, 1996; Yang *et al.,* 1996).

It has recently been reported that catechins, including ECGC, can modulate apoptosis by altering the expression of anti-apoptotic and pro-apoptotic genes in various types of cells. Many studies on the anti-apoptotic effect of EGCG have revealed that EGCG inhibits the expression of pro-apoptotic genes, such as Bax, Bad, and Mdm2, while EGCG increases the expression of anti-apoptotic genes, such as Bcl-2, Bcl-w, and Bcl-x_L (Levites *et al.,* 2002; Weinreb *et al.,* 2003). In the present results, SNP treatment enhanced apoptosis in human lung epithelial cells by decreasing Bcl-2 expression and increasing Bax expression. On the other hand, the cells pre-treated with EGCG showed a noticeable decrease in Bax expression and an increase in Bcl-2 expression.

Activation of downstream caspases, such as caspase-3 or caspase-7, is important for the propagation of the apoptotic signal, and it is considered to be the point of no return in the process leading to cell destruction (Brüne *et al.,* 1998). Caspases have also been shown to activate DNAase leading to chromosomal breakage of DNA during apoptosis (Enari *et al.,* 1998). In the present results, the enzymatic activity of caspase-3 was increased in the SNP-treated cells. Furthermore, the morphologic features of apoptosis resulting from caspase activation were observed. Pre-treatment with EGCG noticeably decreased the activity of caspase-3 in the cells exposed to SNP. EGCG was also reported to inhibit apoptosis and caspase-3 in hepatoma and neuronal cells (Jeong JH *et al.,* 2004; Park HJ *et al.,* 2006). In addition to its direct influence on apoptosis, EGCG possesses free radical scavenging activity and acts as a biological anti-oxidant. In particular, Kelly *et al.* (2001) reported that catechins can scavenge NO (Kelly *et al.,* 2001).

Yang *et al.* (1998) reported that 10 to 100 μM of EGCG is usual experimental concentrations, and 6 or 7 cups of green tea per day is roughly equal to 30 mg/kg EGCG. It is well known that EGCG is permeable to the blood brain barrier (Nakagawa *et al.,* 1997). Thus, 6 or 7 cups of green tea per day might be helpful for the prevention or treatment for excitotoxicity. In the present study, we used 50 μM and 100 μM EGCG, the relevant

concentrations for the experiment.

In the present study, EGCG exerted a protective effect against SNP-induced apoptosis in L-132 human lung epithelial cells by regulating the expression of Bax and Bcl-2 as well as the activity of caspase-3. The results showed that EGCG attenuated the morphological changes caused by SNP-induced apoptosis in L-132 human lung epithelial cells. The underlying mechanisms of suppressing on Bax and enhancing on Bcl-2 of EGCG may be ascribed to the anti-oxidant effects of EGCG as a free radical scavenger of NO. Based on the present results, it can be expected that EGCG may protect against NO-induced lung injuries.

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= 국문초록 =

폐는 질소종류에 다양하게 반응한다. 폐 상피세포는 nitric oxide synthase (NOS)에 의해 L-아르기닌으로부터 합성된 유리기 무기물 가스인 산화질소에 민감하게 반응한다. 산화질소(nitric oxide)는 많은 생물학적 과정의 매개체이지만, 과도한 출현은 다양한 세포 유형의 세포사멸을 일으키기도 한다. 녹차의 주성분이며, 가장 풍부하게 들어있는 (-)-epigallocatechin-3-gallate (ECGC)는 최근에 세포사멸 경로를 조절하는 것으로 알려졌다. EGCG는 항산화, 항염증, 보호기전, 항세포사멸 효과를 보여주고 있다. 이에 본 연구에서는 인간 폐 상피세포에서 EGCG가 sodium nitroprusside (SNP)로 유도된 세포사멸에 대한 보호효과를 연구하였다. 인간의 폐포 유형 II L-132 세포를 가지고, EGCG의 항세포사멸을 확인하기 위해 MTT assay, DAPL staining, TUNEL assay, DNA fragmentation assay, Western blotting으로 분석하였다. 본 실험의 결과 SNP는 인간의 폐 상피 세포인 L-132에서 세포사멸의 형태학적 변화를 유발하였고, 세포사멸 전 단계인 Bax 단백질의 발현을 증가시켰으며, caspase-3의 활성을 증가시켰다. 그러나 EGCE를 처치한 세포에서는 Bax 단백질의 발현을 감소하였고, 반 세포사멸 단백질인 Bcl-2의 발현은 현저하게 증가하여 caspase-3의 활성을 억제하는 결과를 확인하였다. 따라서 본 연구 결과 EGCG는 폐 상피 세포인 L-132에서 SNP로 유도된 세포사멸에 대한 보호효과를 나타냄을 확인할 수 있었다.

중심단어: (-)-Epigallocatechin-3-gallate, Sodium nitroprusside, 산화질소, 인간 폐 상피세포, 세포사멸