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Protective effects of dieckol isolated from *Ecklonia cava* against high glucose-induced oxidative stress in human umbilical vein endothelial cells

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ABSTRACT

The effect of dieckol, one of phlorotannin polyphenol compound purified from *Ecklonia cava* (*E. cava*) against high glucose-induced oxidative stress was investigated using human umbilical vein endothelial cells (HUVECs), which is susceptible to oxidative stress. High glucose (30 mM) treatment induced HUVECs cell death, but dieckol, at concentration 10 or 50 μg/ml, significantly inhibited the high glucose-induced cytotoxicity. Furthermore, treatment with dieckol dose-dependently decreased thiobarbituric acid reactive substances (TBARS), intracellular reactive oxygen species (ROS) generation and nitric oxide level increased by high glucose. In addition, high glucose levels induced the overexpressions of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NF-kB) proteins in HUVECs, but dieckol treatment reduced the overexpressions of these proteins. These findings indicate that dieckol is a potential therapeutic agent that will reduce the damage caused by hyperglycemia-induced oxidative stress associated with diabetes.

1. Introduction

Free radicals or reactive oxygen species (ROS) create oxidative stress, which leads to a variety of pathological lesions and often results in metabolic impairments such as inflammation, aging, cancer, and hypertension (Ames, 1998; Halliwell et al., 1992). Several recent studies have demonstrated that hyperglycemia can cause glucose to undergo autooxidation to generate intermediates that lead to the formation of ROS, nitric oxide (NO), peroxynitrite ($ONOO^-$), and advanced glycation end products (AGE), which cause various complications of diabetes such as nephropathy, retinopathy, and neuropathy. Moreover, hyperglycemia can reduce antioxidant enzyme defenses, thereby allowing ROS to accumulate, resulting in cellular and tissue damage (Giugliano et al., 1996; Baynes and Thorpe, 1999). Antioxidants can prevented pathological damage caused by hyperglycemia-induced oxidative stress associated with diabetes (Yokozawa et al., 2007a,b).

The brown alga *Ecklonia cava* is plentifully produced on Jeju Island in Korea. It is popular in Korea and Japan as a food ingredients, supplement of animal feed and fertilizers, and as a medicine. The total polyphenolic compounds (phlorotannins) in *E. cava* are richer than in other brown algae (Heo et al., 2003, 2005). Phlorotannin components of *E. cava* include phenolic secondary metabolites such as eckol (a closed-chain trimer of phloroglucinol), 6,6′-bieckol (a hexamer), dieckol (a hexamer), phlorofucofuroeckol (a pentamer) and triphlorethol-A that are influential for biological activities (Ahn et al., 2007; Kang et al., 2005a,b). Among these phlorotannins, dieckol is one of the major and active compounds. Its attributes include antioxidant activity (Ahn et al., 2007), antiallergic activity (Le et al., 2008), inhibition of human immunodeficiency virus-1 reverse transcriptase (Ahn et al., 2004), and inhibition of the expression of matrix metalloproteinase-1 (MMP-1) (Joe et al., 2006).

In the present study, we investigated the protective effects of dieckol isolated from *E. cava* against high glucose-induced oxidative stress using human umbilical vein endothelial cells (HUVECs).

2. Materials and methods

2.1. Materials

The brown alga *Ecklonia cava* (*E. cava*) was collected from the coast of Jeju Island, south Korea. Salt, sand and epiphytes were using tap water. Then, the samples were rinsed carefully with fresh water and freeze-dried. Dried alga sample was ground and sifted through a 50-mesh standard testing sieve. All chemicals and

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reagents used were of analytical and obtained from commercial sources.

The UV and FT-IR spectra were recorded on a Pharmacia Biotech Ultrospec 3000 UV/Visible spectrometer and a SHIMAZU 8400s FT-IR spectrometer, respectively. NMR spectra were recorded on a Varian INOVA 400 MHz spectrometer. CD_3OD were used as a solvent for the NMR experiments, and the solvent signals were used as an internal reference. The HPLC was carried out on a YoungLin Instrument HPLC system equipped with a YoungLin acme 9000 UV/VIS detector and Autochrome software using C_{18} column (Waters Spherisorb® DOS-2 RP-18, 4.6 × 250 mm, 5 μm, Waters Co.).

2.2. Isolation of dieckol

The dried *E. cava* powder (500 g) was extracted three times with 80% methanol, and filtered. The filtrate evaporated at 40 °C to obtain the methanol extract, which was dissolved in water, then partitioned with ethyl acetate. The ethyl acetate fraction (45.65 g) was mixed with Celite. The mixed Celite was dried and packed into a glass column, and eluted in the order of hexane, methylene chloride, diethyl ether, and methanol. The diethyl ether fraction (26.69 g) was subjected to Sephadex LH-20 column chromatography using stepwise gradient chloroform/methanol (2/1 to 1/1 to 0/1) solvents system, and then finally purified by reversed-phase HPLC to give compound dieckol (275.8 mg). The purified compound was identified by comparing ^1H and ^{13}C NMR data to the literature report.

2.3. Cell culture

HUVECs were maintained in culture at 37 °C in an humidified atmosphere containing 5% CO_2, in an endothelial cell growth medium-2 EBM-2 supplemented with ascorbic acid, 2% fetal bovine serum (FBS), hydrocortisone, human fibroblast growth factor (hFGF), vascular endothelial growth factor (VEGF), human epidermal growth factor (hEGF), long R insulin-like growth factor-1 (R3-IGF-1), gentamicin sulfate (CA-1000) and heparin as described by the manufacturer (Clonetics, Walkersville, MD, USA).

2.4. Assay of neutral red cell viability

Cell viability was assessed by measuring the uptake of the supravital dye neutral red (Fautz et al., 1991). Cells (4×10^4 cells/well) in wells of 24-well plates were preincubated with glucose (5 mM or 30 mM) for 48 h, and then incubated without or with the indicated concentrations of dieckol for 20 h. Thereafter, the medium was carefully removed from each well, and replaced with 0.5 ml of fresh medium containing 1.14 mM neutral red. After 3 h of incubation, the medium was removed, and then the cells were washed twice with phosphate buffered saline (PBS, pH 7.4). The incorporated neutral red was released from the cells by incubation in the presence of 1 ml of cell lysis buffer [50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 5 mM dithiothreitol (DTT), and 1% (v/v) Triton X-100] containing 1% (v/v) acetic acid and 50% (v/v) ethanol at room temperature for 15 min. To measure dye uptake, the cell lysis products were centrifuged and the absorbance of the supernatant was measured spectrophotometrically at 540 nm.

2.5. Assay of lipid peroxidation

Lipid peroxidation was measured by thiobarbituric acid reactive substances (TBARS) production Fraga et al., 1988. Cells (4×10^4 cells/well) in wells of 24-well plates were preincubated with glucose (5 mM or 30 mM) for 48 h, and then incubated without or with the indicated concentrations of dieckol for 20 h. A 200 μl of each medium supernatant was mixed with 400 μl of TBARS solution then boiled at 95 °C for 20 min. The absorbance at 532 nm was measured and TBARS concentrations were extrapolated from the 1,1,3,3-tetraethoxypropane serial dilution standard curve, TBARS values were then expressed as equivalent nmoles of malondialdehyde (MDA).

2.6. Assay of intracellular ROS levels

Intracellular ROS levels were measured by the dichlorofluorescein assay (Wang and Joseph, 1999). 2′,7′-dichlorodihydrofluorescein diacetate (DCF-DA) can be deacetylated in cells, where it can react quantitatively with intracellular radicals (mainly hydrogen peroxide) to converted into its fluorescent product, DCF, which is retained within the cells. Therefore, DCF-DA was used to evaluate the generation of ROS in oxidative stress. Cells (4×10^4 cells/well) in well of 24-well plates were preincubated with glucose (5 mM or 30 mM) for 48 h, and then incubated without or with the indicated concentrations of dieckol for 20 h, after that the cells were washed with phosphate buffered saline (PBS) and incubated with 5 μM DCF-DA for 30 min at room temperature. Fluorescence was measured using a fluorescence plate reader.

2.7. Assay of nitric oxide (NO) levels

Cells (4×10^4 cells/well) in well of 24-well plates were preincubated with glucose (5 mM or 30 mM) for 48 h, and then incubated without or with the indicated concentrations of dieckol for 20 h. The nitrite accumulation in the supernatant was assessed by Griess reaction (Nath and Powledge, 1997). Each 50 μl of culture supernatant was mixed with an equal volume of Griess reagent [0.1% N-(1-naphthyl)-ethylenediamine, 1% sulfanilamide in 5% phosphoric acid] and incubated at room temperature for 10 min. The absorbance at 550 nm was measured in a microplate absorbance reader, and a series of known concentrations of sodium nitrite was used as a standard.

2.8. Total and nuclear protein extracts

Cells were homogenized with ice-cold lysis buffer containing 250 mM NaCl, 25 mM Tris–HCl (pH 7.5), 1% (v/v) NP-40, 1 mM DTT, 1 mM PMSF, and protease inhibitor cocktail (10 μg/ml aprotinin, 1 μg/ml leupeptin). The cells were then centrifuged at 20,000g for 15 min at 4 °C. The supernatants were used as total protein extracts (Yamabe et al., 2007). For nuclear protein extracts, cells were homogenized with ice-cold lysis buffer containing 50 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 15 mM $CaCl_2$, 1.5 M sucrose, 1 mM DTT, and protease inhibitor cocktail (10 μg/ml aprotinin, 1 μg/ml leupeptin). Then, the cells were centrifuged at 11,000g for 20 min at 4 °C. The supernatants were resuspended with extraction buffer containing 20 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, 25% (v/v) glycerol, 10 mM DTT, and protease inhibitor cocktail (10 μg/ml aprotinin, 1 μg/ml leupeptin). The samples were shaken gently for 30 min and centrifuged at 21,000g for 5 min at 4 °C. The pellets were used as nuclear protein extracts. The total and nuclear protein contents were determined by the Bio-Rad protein kit with BSA as the standard.

2.9. Immunoblotting

iNOS, COX-2 expressions and NF-κB p65 binding activity were determined by western blot analysis (Yamabe et al., 2007). Total protein (30 μg) for iNOS, COX-2 protein levels and nuclear protein (30 μg) for NF-κB were electrophoresed through 10% sodium dodecyl sulfate–polyacrylamide gel. Separated proteins were transferred electrophoretically to a pure nitrocellulose membrane,

blocked with 5% skim milk solution for 2 h, and then incubated with primary antibodies overnight at 4 °C. After washing of the blots, they were incubated with goat anti-rabbit or goat anti-mouse IgG HRP conjugated secondary antibody for 2 h at room temperature. Each antigen–antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with LAS-1000 plus (FUJIFILM, Tokyo, Japan). Band densities were determined by an image analyzer ATTO densitograph (ATTO, Tokyo, Japan) and normalized to β-actin for total protein and nuclear protein.

2.10. Data and statistical analysis

The data are represented as mean ± SD. The statistical analysis was performed using SAS software. The values were evaluated by one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range tests.

3. Results

3.1. Structure elucidation of dieckol

Dieckol: amorphous powder, ^{1}H NMR (400 MHz, methanol-d_4) δ 6.15 (1H, s), 6.13 (1H, s), 6.09 (1H, d, 2.9 Hz), 6.06 (1H, d, 2.9 Hz), 6.05 (1H, d, 2.9 Hz), 5.98 (1H, d, 2.8 Hz), 5.95 (1H, d, 2.8 Hz), 5.92 (3H, m); ^{13}C NMR (100 MHz, methanol-d_4) δ 161.8, 160.1, 157.8, 155.9, 154.5, 152.4, 147.3, 147.2, 147.1, 146.9, 144.3, 144.1, 143.4, 143.3, 138.6, 138.5, 126.5, 126.2, 125.6, 125.5, 124.9, 124.6, 124.5, 99.9, 99.7, 99.5, 99.4, 97.6, 96.2, 95.8, 95.7, 95.3; FAB-MS m/z 742 $[M]^+$. It was definitely elucidated as dieckol (Fig. 1).

3.2. Neutral red cell viability

Fig. 2 shows the effect of dieckol on cell viability in HUVECs treated with high glucose of 30 mM was examined by neutral red assay. When HUVECs were treated with 30 mM glucose for 48 h, the cell viability significantly decreased. Cell viability was decreased to 36.6% in 30 mM glucose-treated HUVECs, but, dieckol protected against the cellular damage induced 30 mM glucose in a dose-dependent manner. Especially, treatment with 50 μg/ml of dieckol together with high glucose resulted in significant increase to 79.7% in cell viability.

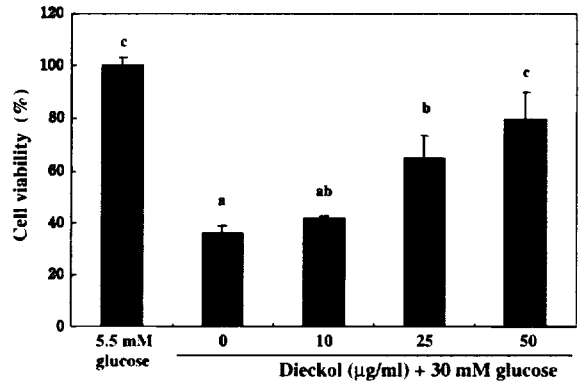

Fig. 2. Effect of dieckol on cell viability in high-glucose-treated HUVECs. Cells in wells of 24 well plates (4×10^4 cells/well) were preincubated with 5.5 or 30 mM glucose for 48 h, and then incubated in the absence of dieckol or 10, 25, or 50 μg/ml dieckol for 20 h. The use of 5.5 mM glucose represents normal glucose and 30 mM represents high glucose. Each value is expressed as mean ± SD ($n = 3$). $^{a-c}$Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

3.3. Lipid peroxidation

As shown in Fig. 3, the effect of dieckol on lipid peroxidation in high glucose-treated HUVECs was determined by measuring TBARS, a lipid peroxidation product. When HUVECs were incubated with 30 mM glucose for 48 h, TBARS was significantly increased in comparison to the cells treated with 5.5 mM glucose. Treatment with 50 μg/ml of dieckol together with high glucose significantly inhibited TBARS formation, indicating protection against lipid peroxidation. When the cells were treated with 50 μg/mL of dieckol, TBARS was significantly decreased by 0.41 nmol MDA.

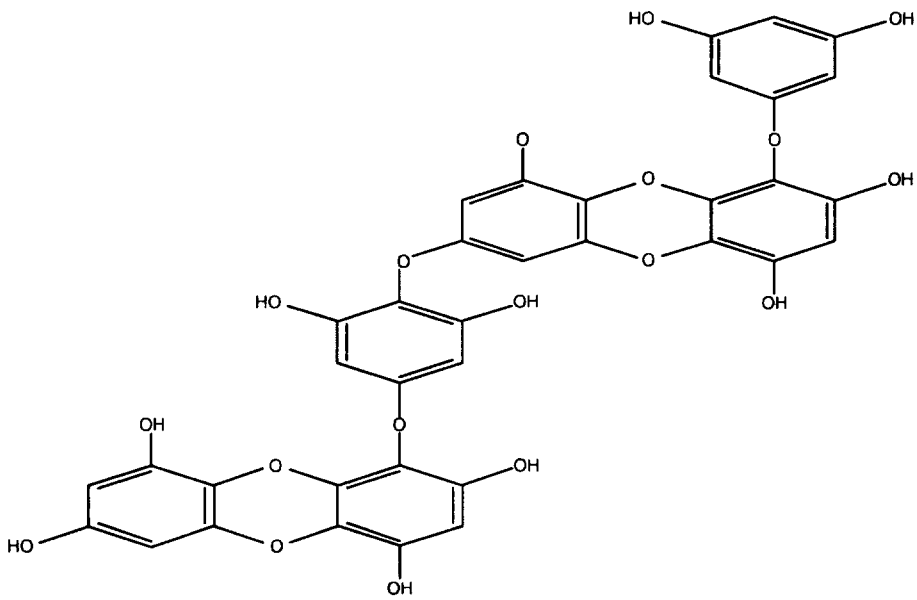

Fig. 1. Chemical structure of dieckol isolated from *Ecklonia cava*.

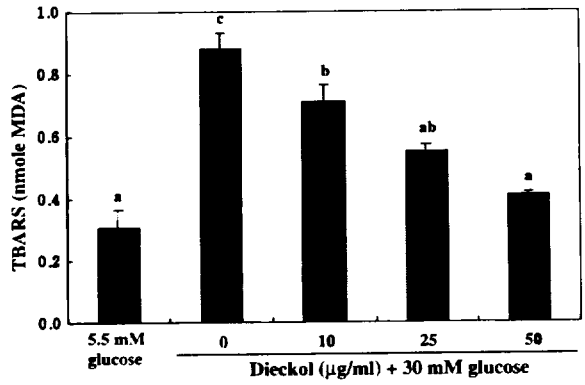

Fig. 3. Effect of dieckol on TBARS generation in high-glucose-treated HUVECs. Cells in wells of 24 well plates (4×10^4 cells/well) were preincubated with glucose and incubated in the absence or presence of dieckol as described in the legend to Fig. 2. Each value is expressed as mean ± SD ($n = 3$). $^{a-d}$Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

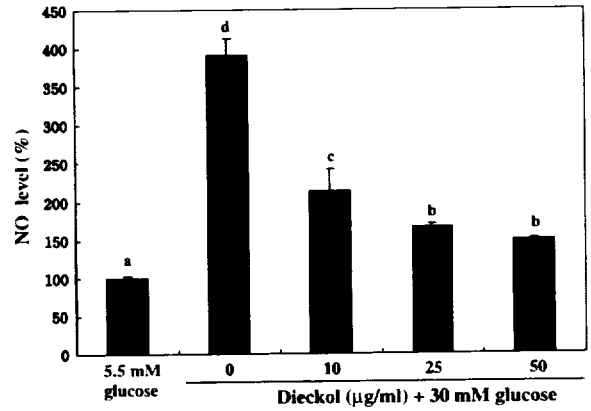

Fig. 5. Effect of dieckol on NO level in high-glucose-treated HUVECs. Cells in 24 well plates (4×10^4 cells/well) were preincubated with glucose and incubated in the absence or presence of dieckol as described in the legend to Fig. 2. Each value is expressed as mean ± SD ($n = 3$). $^{a-c}$Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

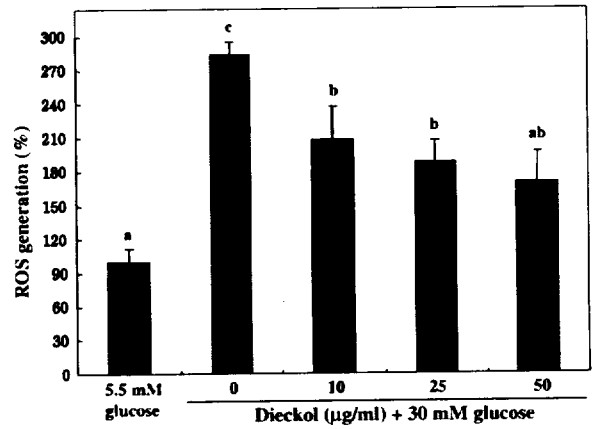

Fig. 4. Effect of dieckol on intracellular ROS generation in high-glucose-treated HUVECs. Cells in 24 well plates (4×10^4 cells/well) were preincubated with glucose and incubated in the absence or presence of dieckol as described in the legend to Fig. 2. Each value is expressed as mean ± SD ($n = 3$). $^{a-d}$Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

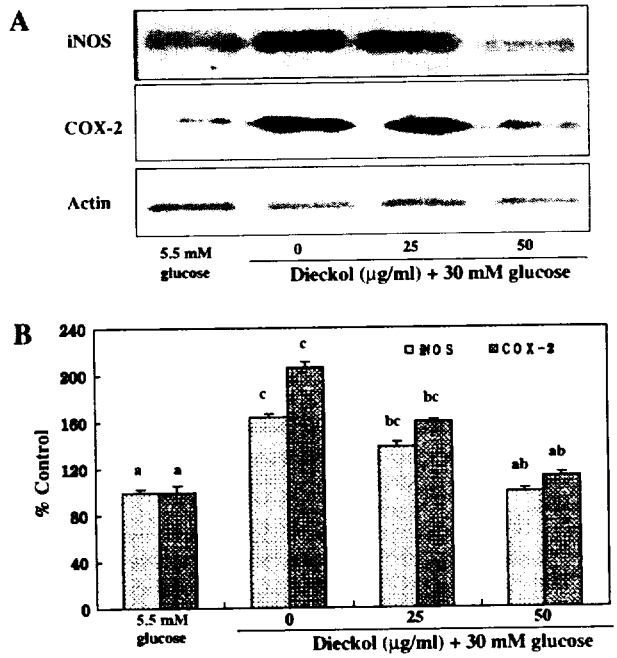

Fig. 6. Effects of dieckol on iNOS and COX-2 expressions. (A) Equal amounts of cell lysates (30 μg) were subjected to electrophoresis and analyzed for iNOS and COX-2 expressions by Western blot. Actin was used as an internal control. (B) HUVECs were preincubated with glucose and incubated in the absence or presence of dieckol as described in the legend to Fig. 2. Each value is expressed as mean ± SD ($n = 3$). $^{a-c}$Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

3.4. Intracellular ROS generation

As shown in Fig. 4, the generation of intracellular ROS in HUVECs increased significantly after treatment with 30 mM glucose compared with 5.5 mM glucose. When HUVECs were cultured with 30 mM glucose, intracellular ROS increased significantly to 284.1%. However, treatment of dieckol decreased dose-dependently the ROS level in the cells induced with 30 mM glucose. Especially, treatment with 50 μg/ml of dieckol resulted in a significant decrease in intracellular ROS to 170.4%. Dieckol significantly decreased the elevated ROS level induced by high glucose.

3.5. NO generation

As shown in Fig. 5, the level of NO in HUVECs was significantly elevated by 30 mM glucose treatment compared with 5 mM glucose treatment. However, NO levels in dieckol treated cells significantly decreased and this effect was concentration dependent. The level of NO in HUVECs treated 30 mM glucose is 390.6%, but treatment with 50 μg/ml of dieckol together with high glucose exposure resulted in significant decrease in intracellular NO to 147.8%. dieckol scavenged NO produced by high glucose-induced oxidative stress.

3.6. iNOS and COX-2 expressions

To determine whether dieckol inhibits high glucose-induced overexpressions of iNOS and COX-2 proteins, dieckol concentrations of 25 and 50 μg/ml were added to HUVECs. As shown in

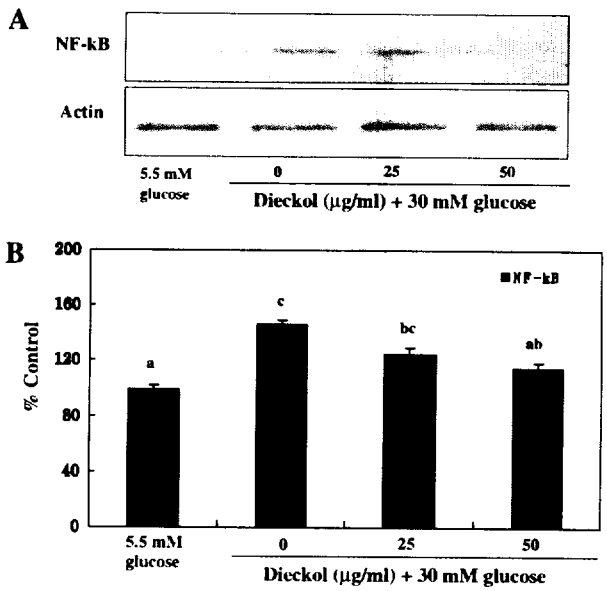

Fig. 7. Effect of dieckol on NF-κB activity. (A) Equal amounts of cell lysates (30 μg) were subjected to electrophoresis and analyzed for NF-κB activity as described in the legend to Fig. 6A. (B) HUVECs were preincubated with glucose and incubated in the absence or presence of dieckol as described in the legend to Fig. 2. Each value is expressed as mean ± SD ($n = 3$). $^{a-c}$Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test.

Fig. 6, the levels of iNOS and COX-2 expressions were clearly higher in 30 mM glucose-treated HUVECs than in 5.5 mM glucose-treated HUVECs. However, these expression levels by treatment with dieckol were reduced markedly. A 50 μg/ml of dieckol showed each maximum inhibitory effect in iNOS and COX-2 expressions. Actin was used as a house-keeping control gene.

3.7. NF-κB activity

Specific DNA binding of NF-κB showed that high glucose treatment to HUVECs enhanced NF-κB activation (Fig. 7). The levels of NF-κB activity was clearly higher in 30 mM glucose-treated HUVECs than in 5.5 mM glucose-treated HUVECs. However, when dieckol concentrations of 25 and 50 μg/ml were added to HUVECs, NF-κB activity decreased markedly. 50 μg/ml of dieckol showed a maximum inhibitory effect of NF-κB activity. Actin was used as a house-keeping control gene.

4. Discussion

Oxidative stress induced by increase of hyperglycemia causes diabetes-associated pathological damage (Fraga et al., 1988; Uemura et al., 2001). Thus, to reduce the risk of pathological damage such as diabetes, attenuation of oxidative stress induced by hyperglycemia is important. There is great interest in identifying antioxidant compounds that do not exhibit side effects or toxicity. Marine algae are rich in the biological polyphenolic compounds called phlorotannins. The brown alga *E. cava* possesses various phlorotannins such as eckol, 6,6′-bieckol, dieckol, phlorofucofuroeckol and triphlorethol-A. In particular, *E. cava* dieckol exhibits strong antioxidant activity (Ahn et al., 2007; Kim et al., 2004; Shibata et al., 2008). Presently, we investigated the protective effects of dieckol isolated from *E. cava* against high glucose-induced oxidative stress using human umbilical vein endothelial cells (HUVECs). Hyperglycemia is thought to be an important regulator of vascular lesion development. Hyperglycemia-induced endothelial cell dysfunctions accelerate the process of atherothrombotic complications. Vascular disorders are thought to participate in the pathogenesis of ahterosclerosis. Therefore, it suggested that high glucose-induced oxidative stress from HUVEC is important in determining the character of diabetic complication.

To measure neutral red cell viability, the MTT assay was examined. Exposure of HUVECs to high glucose resulted in a significant decrease of cell viability (Fig. 2). However, dieckol treatment inhibited cell death, suggesting that dieckol protect HUVECs from high glucose-induced cytotoxicity.

Lipid peroxidation may be a form of cell damage that is mediated by free radicals (Sevanian and Hochstein, 1985). Presently, high glucose induced lipid peroxidation in HUVECs and dieckol inhibited TBARS formation effectively (Fig. 3). One of the serious consequences of lipid peroxidation is damage to biomembranes such as mitochondrial and plasma membranes. During lipid peroxidation, low molecular-weight end products, likely malondialdehyde (MDA), are formed by oxidation of polyunsaturated fatty acids. These end products can react with two molecules of thiobarbituric acid to give a pinkish red chromogen. The presently-demonstrated protective action of dieckol on TBARS formation can be attributed to its antiperoxidative effect.

High ROS levels induce oxidative stress, which can result in a variety of biochemical and physiological lesions. Such cellular damage often impairs metabolic function, and leads to cell death (Finkel et al., 2000). Our results showed that treatment of HUVECs with 30 mM glucose significantly increased the intracellular ROS level. However, dieckol inhibited the high glucose-induced ROS generation (Fig. 4). Previously, we reported that dieckol isolated from *E. cava* inhibits hydrogen peroxide-mediated DNA damage and formation of harmful free radicals (Ahn et al., 2007). The previous and present results indicate that dieckol has ROS scavenging activity.

High glucose treatment leads to overproduction of NO and superoxide anion ($O_2^{\cdot-}$) Du et al., 1999; Ishii et al., 2001; Suarez-Pinzon et al., 2001. NO and $O_2^{\cdot-}$ separately cause ischemic renal injury and, collectively, these radicals work together to bring about further damage. The toxicity and damage caused by NO and $O_2^{\cdot-}$ are multiplied as they react to produce reactive $ONOO^-$, which leads to serious toxic reactions, including SH-group oxidation, protein tyrosine nitration, lipid peroxidation and DNA modifications (Radi et al., 1991; Moncada et al., 1991). In addition, NO and $O_2^{\cdot-}$ induce highly reactive oxidative damage associated with diabetes (Beckman et al., 1990; Mandrup-Poulsen et al., 1990). In the present study, we found that dieckol scavenged NO produced by high glucose-induced oxidative stress (Fig. 5). The findings suggest that dieckol might confer important protection against the oxidative stress induced by hyperglycemia.

Proinflammatory enzymes including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) produce a large amount of NO, which influences many chronic diseases associated with oxidative stress. Generally, COX-2 is barely detectable under normal physiological conditions. Presently, high glucose induced overexpression of COX-2 protein, which was reduced by dieckol in a dose-dependent manner (Fig. 6). However, the level of NO released from iNOS under stimulation, such as high glucose conditions, as in the present study, or under inflammatory conditions, is significantly raised and forms the potent free radical (Spencer et al., 1997). Therefore, an increase in iNOS production has been proposed to be responsible for multiple organ dysfunctions (Thiemermann et al., 1995). In our study, high glucose induced the overexpression of COX-2 and iNOS, but dieckol inhibited these potent expressions (Fig. 6). These findings imply that dieckol alleviates oxidative stress by inhibiting the expression of iNOS and COX-2 enzymes.

Nuclear factor-kappa B (NF-κB p65), an oxidative stress responsive transcription factor, plays an important role in the mechanism

of cell injury and in the induction of iNOS and COX-2, which are both expressed as a result of NF-κB activation (Baeuerle and Baltimore, 1996; Han et al., 2004). In particular, NF-κB is activated in cells cultured under conditions of high glucose concentrations (Du et al., 1999; Hattori et al., 2000). NF-κB has important roles in the expression of a variety of genes involved in inflammatory responses and in apoptosis in multiple tissues and cell types (Baldwin, 1996). NF-κB activation is inhibited by antiinflammatory agents and antioxidant inhibitors (Bayon et al., 1999; Shishodia et al., 2003). We presently observed that dieckol inhibited high glucose-induced NF-κB activation in HUVECs (Fig. 7), indicating that NF-κB reduction by dieckol may contribute to the attenuation of intracellular oxidative stress.

In our study, HUVEC damage occurred as a result of high glucose induced overproduction of ROS, lipid peroxidation products, and NO. In addition, high glucose levels induced the overexpression of iNOS and COX-2, and enhanced NF-κB activation in HUVECs. Dieckol protected cells against the oxidative stress by reducing ROS generation and inhibiting production of iNOS and COX-2, and by reducing NF-κB activation. Taken together, these results suggest that dieckol may contribute to the prevention of oxidative stress-related diseases including diabetes.

Polyphenolic compounds from marine algae have strong antioxidative activities on free radicals (Ahn et al., 2007; Kang et al., 2003, 2005a,b; Kim et al., 2004; Kang et al., 2003). Also, the previous study indicated that polyphenols are electron-rich compounds, which are prone to enter into efficient electron-donation reactions and produce phenoxyl radicals ($PhO^{\cdot}$) as intermediates in the presence of oxidizing agents. $PhO^{\cdot}$ are stabilized by resonance delocalization of the unpaired electron to the *ortho* and *para* positions of the ring. In addition to the resonance stability, $PhO^{\cdot}$ can also be stabilized by hydrogen bonding with an adjacent hydroxyl group. $PhO^{\cdot}$ also undergo dimerization ("phenol coupling") to produce new C–C or C–O linkage (Larson, 1997). This intrinsic stability of polyphenol structures and their potency as an electron donor might be related to the antioxidative activity of dieckol.

In conclusion, we demonstrate the promising therapeutic potential of dieckol for pathological conditions associated with hyperglycemia-induced oxidative stress that occurs in diabetes.

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