Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 459–466
Journal of Nutritional Biochemistry

Piceatannol attenuates hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells by blocking down-regulation of Bcl-X_L and activation of JNK

Hyo Jin Kim[a,1], Ki Won Lee[b,1], Mi-Sung Kim[a], Hyong Joo Lee[a,*]

[a]*Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Republic of Korea*
[b]*Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Republic of Korea*

Received 31 January 2007; received in revised form 22 May 2007; accepted 1 June 2007

Abstract

There is mounting evidence implicating the accumulation of intracellular reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the pathogenesis of neurodegenerative disorders, including Alzheimer's disease. Recently, considerable attention has been focused on identifying naturally occurring antioxidants that are able to reduce excess ROS and RNS, thereby protecting against oxidative stress and neuron death. The present study investigated the possible protective effects of piceatannol (*trans*-3,4,3′,5′-tetrahydroxystilbene), which is present in grapes and other foods, on hydrogen-peroxide- and peroxynitrite-induced oxidative cell death. PC12 rat pheochromocytoma (PC12) cells treated with hydrogen peroxide or SIN-1 (a peroxynitrite-generating compound) exhibited apoptotic death, as determined by nucleus condensation and cleavage of poly(ADP-ribose)polymerase (PARP). Piceatannol treatment attenuated hydrogen-peroxide- and peroxynitrite-induced cytotoxicity, apoptotic features, PARP cleavage and intracellular ROS and RNS accumulation. Treatment of PC12 cells with hydrogen peroxide or SIN-1 led to down-regulation of Bcl-X_L and activation of caspase-3 and -8, which were also inhibited by piceatannol treatment. Hydrogen peroxide or SIN-1 treatment induced phosphorylation of the c-Jun-N-terminal kinase (JNK), which was inhibited by piceatannol treatment. Moreover, SP600125 (a JNK inhibitor) significantly inhibited hydrogen-peroxide- and peroxynitrite-induced PC12 cell death, revealing inactivation of the JNK pathway as a possible molecular mechanism for the protective effects of piceatannol against hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells. Collectively, these findings suggest that the protective effect of piceatannol against hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells is associated with blocking the activation of JNK and the down-regulation of Bcl-X_L.

Keywords: Piceatannol; Hydrogen peroxide; Peroxynitrite; Apoptosis; c-Jun N-terminal kinase; Bcl-X_L

1. Introduction

Neurodegenerative diseases have diverse clinical implications and etiologies. Epidemiological and experimental studies have identified a wide range of risk factors associated with neurodegenerative disorders, such as age, genetic defects, excitotoxicity, oxidative stress, abnormalities of antioxidant enzymes, deficiencies in neurotransmitters, metabolic toxicity, autoimmunity and hypertension [1]. Alzheimer's disease (AD) is a typical neurodegenerative disorder characterized by progressive degeneration and loss of neurons in the brain, which have been attributed to the appearance of neurofibrillary tangles and senile plaques, the two neuropathological hallmarks of AD [2]. AD is now recognized as being accountable for redox regulation involving reactive oxygen species (ROS) and reactive nitrogen species (RNS). It is also likely that these risk factors, acting over time, increase the propensity for the occurrence of inflammatory events within the central nervous system, which, in turn, increase the production of ROS and RNS, which then contribute to the neurodegenerative processes [3].

Oxidative stress thus occurs when the production of free radicals increases, scavenging of free radicals or repair of

* Corresponding author. Tel.: +82 2 880 4860; fax: +82 2 873 5095.
E-mail address: leehyjo@snu.ac.kr (H.J. Lee).
[1] These authors contributed equally to this work.

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.06.001

oxidatively damaged macromolecules decreases, or both. Hydrogen peroxide is a representative ROS that is produced during the redox process and was recently considered to act as a messenger in intracellular signaling cascades [4,5]. Although not reactive per se, hydrogen peroxide forms the highly reactive hydroxyl radical by Fenton's reaction in the presence of a transition-metal ion. The hydroxyl radical reacts rapidly with almost every cellular macromolecule — including DNA, lipids and proteins — to produce both functional and structural alterations therein. In addition to oxidative stress, nitrosative stress caused by the increased intracellular accumulation of RNS, such as nitric oxide (NO) and peroxynitrite [3-(4-morpholinyl) sydnonimine hydrochloride], has been implicated in many pathophysiological processes, such as brain ischemia, inflammation and neurodegeneration, including AD. Peroxynitrite, which is the product of the reaction between NO and superoxide, is involved in the oxidation of proteins, lipids and nucleic acids in AD [6].

Both ROS and RNS can cause cell death via apoptosis in many cell types, and such proapoptotic effects can be blocked or delayed by a wide variety of antioxidants or antiapoptotic proteins [3,7]. Recently, consider attention has been focused on a wide array of nonvitamin antioxidants, such as polyphenolic components that are able to attenuate intracellular ROS and RNS accumulation, thereby protecting against oxidative damage [8]. Piceatannol (*trans*-3,4,3′,5′-tetrahydroxystilbene) is a polyphenol present in grapes and wine [9] and in the seeds of *Euphorbia lagascae* [10]. Previous studies showed that piceatannol exhibited anticancer activity [9,11], but the protective effect of piceatannol against oxidative and nitrosative neuron cell death has not been reported. As part of our program to evaluate the neuroprotective activity of piceatannol, we sought to determine whether piceatannol could protect PC12 rat pheochromocytoma (PC12) cells from oxidative and nitrosative cell death induced by hydrogen peroxide and peroxynitrite.

2. Materials and methods

2.1. Chemicals

Hydrogen peroxide was obtained from Junsei Chemical (Tokyo, Japan). SIN-1, poly-D-lysine, 2′,7′-dichlorofluorescein diacetate (DCF-DA), dihydrorhodamine 123 (DHR123), 4,6-diamidino-2-phenylindole (DAPI) and 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma Chemical (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum and horse serum were obtained from GIBCO BRL (Grand Island, NY, USA). Anti-poly (ADP-ribose)polymerase (PARP), anti-Bcl-X_L, anti-c-Jun-N-terminal kinase (JNK) and anti-phosphorylated JNK antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). An anti-β-actin antibody was purchased from Sigma Chemical. All other chemicals used were of analytical grade.

2.2. Cell culture

PC12 cells were kindly provided by Dr. Y.-J. Surh at Seoul National University and were grown in DMEM supplemented with 10% heat-inactivated horse serum and 5% fetal bovine serum at 37°C in a humidified atmosphere of 10% CO_2 and 90% air.

2.3. Determination of cell viability

The MTT assay provides a sensitive measurement of the normal metabolic status of cells, particularly that of mitochondria, which reflects early cellular redox changes. Cells (2×10^3) were cultured in a 96-well plate for 24 h. The cells were treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 24 h in the presence or absence of piceatannol (10 and 20 μM, respectively) and were then treated with the MTT solution (final concentration, 1 mg/ml) for 2 h. The dark-blue formazan crystals formed in intact cells were dissolved in DMSO, and the absorbance at 570 nm was measured with a microplate reader. Results are expressed here as the percentage of MTT reduction relative to the absorbance of control cells.

2.4. Measurement of nuclear condensation

Cells (5×10^3) were cultured in a 24-well plate for 24 h and were then incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 30 min in the presence or absence of piceatannol (10 and 20 μM). The cells were washed with PBS and fixed with 70% ethanol for 20 min. The fixed cells were washed with PBS and stained with the DNA-specific fluorochrome DAPI (1 μg/ml). Following 10 min of incubation, the cells were washed with PBS, and the plates were observed under a fluorescence microscope (Olympus Optical, Japan). Apoptotic cells were determined by the morphological changes after staining with DAPI. Piceatannol-treated cells were expressed as percent degradation compared to hydrogen peroxide or SIN-1-treated cells. Data are represented as means±S.D. of the apoptotic cells calculated from three separate experiments.

2.5. Western blot analysis

Cells (2×10^5) were cultured in a 60-mm plate for 24 h, and the cells were then treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for the indicated time in the presence or absence of piceatannol (10 and 20 μM). Cells were washed with PBS and collected with 100 μl of lysis buffer [20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml leupeptin]. The protein concentration was determined using the BCA protein assay kit. Protein samples were electrophoresed through a 12.5% SDS-polyacrylamide gel. Proteins on the gel were transferred onto a polyvi-

nylidene difluoride transfer membrane that was blocked with 5% skim milk containing 0.5 mM Tris–HCl (pH 7.5), 150 mM NaCl and 0.05% Tween-20 for 2 h at room temperature. The membrane was subsequently incubated with the primary antibody. After five washes with PBST (0.1% Tween-20 in PBS), the blots were incubated with horseradish-peroxidase-conjugated secondary antibodies in PBST with 5% skim milk at a 1:5000 dilution for 2 h at room temperature. The blots were then again washed five times in PBST buffer. Blots were developed using the enhanced chemiluminescence (ECL) detection method by immersing them for 5 min in a mixture of ECL reagents A and B at the ratio 1:1 and exposing them to Kodak film for a few minutes.

2.6. Assessment of caspase-3 and -8 activities

Cells (1×10^5) were cultured in a 24-well plate for 24 h, and the cells were then treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 12 h in the presence or absence of piceatannol (10 and 20 μM). The cells were washed with PBS and lysed for 30 min at 4°C. Cell lysates were kept on ice for 30 min and then centrifuged at 12,000×*g* for 20 min at 4°C. The protein concentrations in the supernatants were determined using the BCA protein assay kit. The enzymatic activities of caspase-3 and -8 were assayed with 200 mM Ac-DEVD-MCA fluorogenic substrates in the assay buffer. Fluorescence was measured at 460 nm using a fluorescence spectrofluorometer, with excitation at 360 nm.

2.7. Measurement of intracellular ROS and RNS accumulation

DCF-DA can be deacetylated in cells, where it can react quantitatively with intracellular radicals (mainly hydrogen peroxide) to be converted into its fluorescent product, DCF, which is retained within the cells. Subsequent spectrophotometry measurements using DHR123 thus provide an index of cell cytosolic oxidation and generation of peroxynitrite. The peroxynitrite-dependent oxidation of DHR123 to rhodamine 123 was measured as described previously. We measured the intracellular production of ROS and RNS using DCF-DA and DHR123 assays, respectively. Cells (1×10^5) were cultured in a 24-well plate for 24 h, and the cells were then treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 30 min in the presence or absence of piceatannol (10 and 20 μM). The cells were rinsed with PBS solution, and 10 mM DCF-DA/DHR123 was loaded. After 15 min of incubation at 37°C, the cells were examined at 530 nm with a confocal microscope equipped with a fluorescence spectrophotometer (F-4500, Hitachi, Tokyo, Japan), with excitation at 485 nm.

2.8. Statistical analysis

When necessary, data are expressed as means±S.D., and the Student's *t* test was used in statistical analysis for single comparison. $P<.05$ was used as the criterion for statistical significance.

3. Results

3.1. Piceatannol provided stronger protection than resveratrol against hydrogen-peroxide- and peroxynitrite-induced cytotoxicity

PC12 cells were exposed to hydrogen peroxide or SIN-1 (a generator of peroxynitrite) in the presence of piceatannol or resveratrol for 24 h, and then, cell viability was evaluated using the conventional MTT reduction assay. Hydrogen peroxide at 150 μM and SIN-1 at 1.5 mM decreased the cell viability, and this effect was dose-dependently attenuated by treatment with piceatannol (Fig. 1). The compounds at these concentrations alone did not cause any apparent cytotoxicity (data not shown).

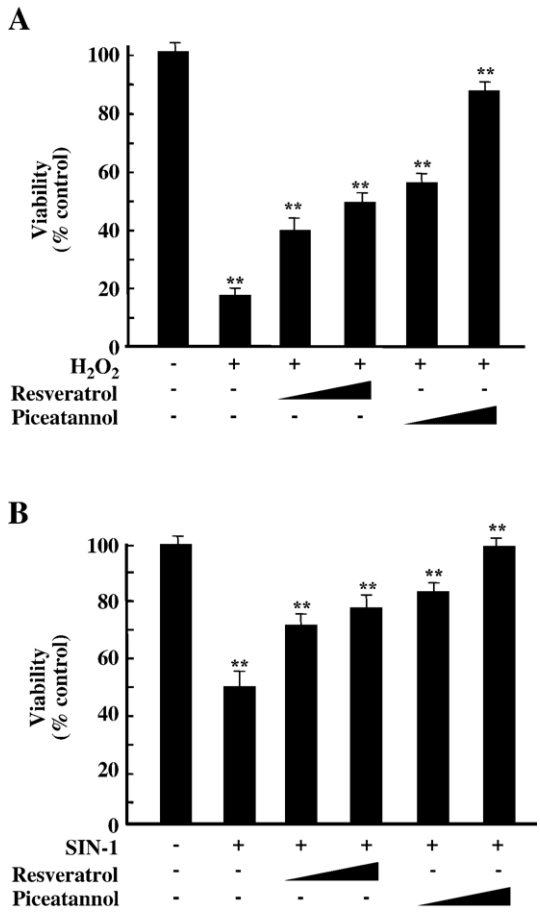

Fig. 1. Protective effects of piceatannol and resveratrol on the cytotoxic effects of hydrogen peroxide (A) and SIN-1 (B) in PC12 cells. PC12 cells were incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 24 h in the presence or absence of piceatannol (10 and 20 μM, respectively). The cell viability was determined using the MTT reduction assay as described in Section 2. Data are mean±S.D. values for three independent experiments. (**$P<.01$, as compared with cells treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 alone.)

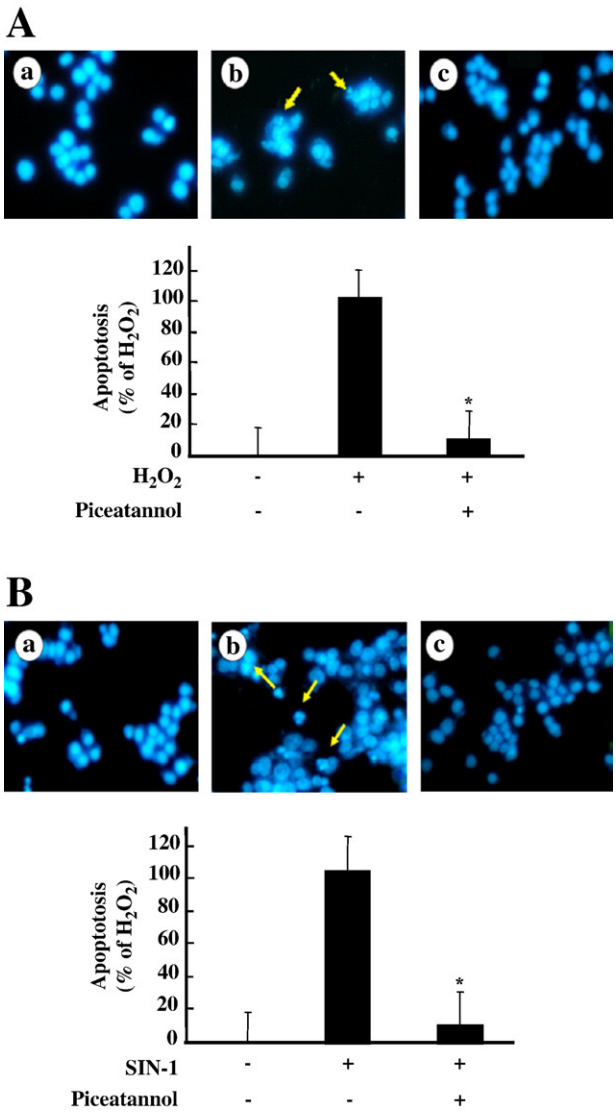

Fig. 2. Effects of piceatannol on nuclear condensation in PC12 cells induced by hydrogen peroxide (A) and SIN-1 (B). The nuclear morphology of cells was examined by fluorescence microscopy as described in Section 2. (A) (a) Control, (b) 150 μM hydrogen peroxide, (c) 150 μM hydrogen peroxide and 10 μM piceatannol. (B) (a) Control, (b) 1.5 mM SIN-1, (c) 1.5 mM SIN-1 and 10 μM piceatannol. Condensed and segmented nuclei are indicated by arrows. Apoptosis is expressed as percent inhibition compared to H_2O_2- or SIN-1-treated cells. Data are mean±S.D. values for three independent experiments. (*P<.05, as compared with cells treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 alone.)

3.2. Piceatannol attenuated hydrogen-peroxide- and peroxynitrite-induced apoptosis

Cells undergoing apoptosis display a distinct pattern of structural changes in the nucleus and cytoplasm, including rapid blebbing of the plasma membrane and nuclear disintegration. We next assessed apoptosis in PC12 cells by examining nuclear morphological changes using the DNA-specific fluorescent dye DAPI. Treatment with hydrogen peroxide (Fig. 2A,b) or SIN-1 (Fig. 2B,b) for 24 h increased the proportion of condensed and fragmented nuclei in a dose-dependent manner (data not shown). The fragmentation of nuclei induced by hydrogen peroxide and peroxynitrite was significantly attenuated by treatment with 10 μM piceatannol (Fig. 2A,c and B,c).

3.3. Piceatannol inhibited hydrogen-peroxide- and peroxynitrite-induced cleavage of PARP

Cleavage of PARP is associated with the induction of apoptosis in neurons [12]. To examine the hydrogen-peroxide- and peroxynitrite-induced apoptotic cell death, we next investigated whether hydrogen peroxide or peroxynitrite induces the cleavage of PARP. Treatment with hydrogen peroxide or SIN-1 for 24 h caused cleavage of the PARP, and this was inhibited by piceatannol treatment (Fig. 3).

3.4. Piceatannol inhibited hydrogen-peroxide- and peroxynitrite-induced down-regulation of Bcl-X_L

The intracellular concentration of the antiapoptotic protein Bcl-2 represents a molecular indicator of whether a cell dies or survives [13]. Bcl-X_L, which is a protein that is structurally and functionally analogous to Bcl-2, blocks apoptosis induced by a wide array of death signals [14]. Because Bcl-2 expression is barely detectable in PC12 cells, we used Western blot analysis to measure the concentration

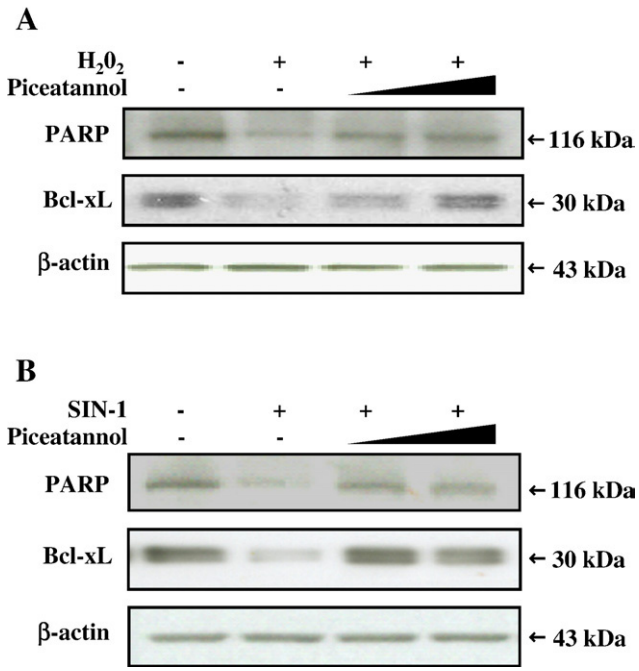

Fig. 3. Effects of piceatannol on cleavage of PARP and degradation of Bcl-X_L induced by hydrogen peroxide (A) and SIN-1 (B). PC12 cells were incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 24 h in the presence or absence of piceatannol (10 and 20 μM). Western blot analyses were performed for PARP, Bcl-X_L and β-actin as described in Section 2. Data are representative of two independent experiments that gave similar results.

of Bcl-X_L. Treatment of PC12 cells with hydrogen peroxide or SIN-1 for 24 h decreased the expression of Bcl-X_L, which was blocked by piceatannol treatment (Fig. 3). Equal loading of proteins was verified by probing the replicate blots for β-actin.

3.5. Piceatannol inhibited hydrogen-peroxide- and peroxynitrite-induced activation of caspase-3 and -8

The complex cascade of caspase activation is specific to apoptosis, in which caspase-3 and -8 have been shown to play pivotal roles [15]. Although previous studies have shown that caspase-3 is activated in response to amyloid beta in PC12 cells, it has not been clarified whether hydrogen peroxide or peroxynitrite activates caspase-3 and -8. We found that exposure with hydrogen peroxide or SIN-1 resulted in up-regulation of caspase-3 and -8 activities, as demonstrated using the specific fluorogenic peptide substrates for caspases (Fig. 4). The hydrogen-peroxide- and peroxynitrite-induced activations of caspase-3 and -8 were significantly inhibited by treatment with 10 μM piceatannol (Fig. 4).

3.6. Piceatannol prevented hydrogen-peroxide- and peroxynitrite-induced ROS and RNS accumulation

The treatment of PC12 cells with hydrogen peroxide or peroxynitrite induces significant cytosolic and mitochondrial production of ROS and RNS, which can be measured using DCF-DA and DHR123 assays. Exposure of PC12 cells to hydrogen peroxide or SIN-1 resulted in six- to sevenfold increases in cellular DCF-DA and DHR123 fluorescence intensity (Fig. 5). The changes in fluorescence intensity revealed that the pretreatment of 10 μM piceatannol significantly attenuated intracellular ROS and RNS accumulation.

3.7. Piceatannol blocked hydrogen-peroxide- and peroxynitrite-induced phosphorylation of JNK

Activation of JNK has been implicated in neuron death caused by a wide variety of toxicants [12]. To explore the molecular mechanisms responsible for the protective effect of piceatannol against hydrogen-peroxide- and peroxynitrite-induced oxidative cell death, we assessed phosphorylation of

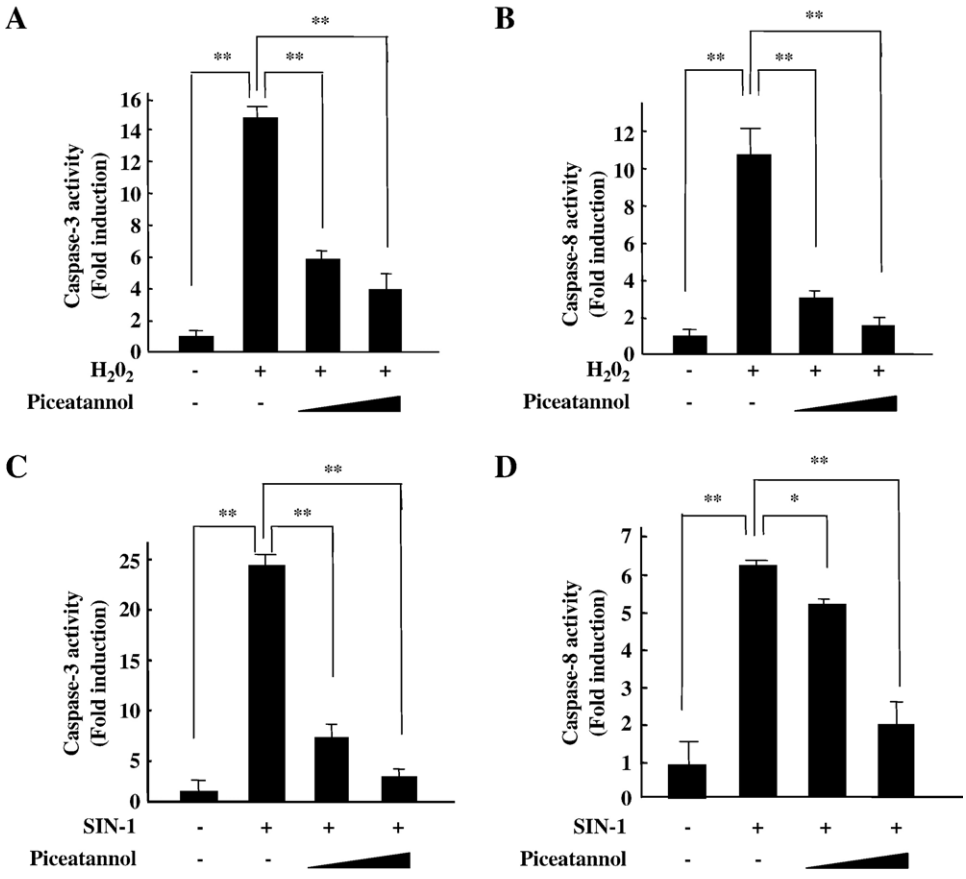

Fig. 4. Effects of piceatannol on activation of caspase-3 and -8 induced by hydrogen peroxide (A) and SIN-1 (B). PC12 cells were incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 12 h in the presence or absence of piceatannol (10 and 20 μM). The caspase-3 and -8 activities were measured using the colorimetric substrate as described in Section 2. Enzyme activity is expressed relative to that in vehicle-treated controls. (*P<.05 and **P<.01, as compared with cells treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 alone.)

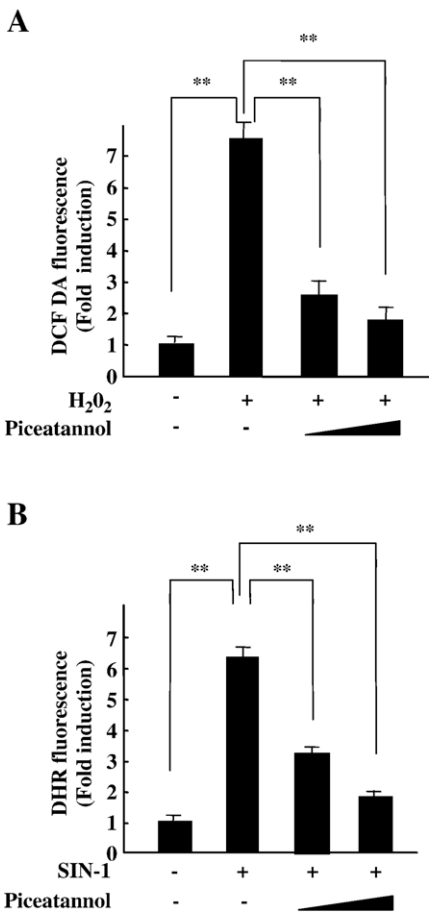

Fig. 5. Effects of piceatannol on accumulation of intracellular ROS and RNS induced by hydrogen peroxide (A) and SIN-1 (B). PC12 cells were incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 30 min in the presence or absence of piceatannol (10 and 20 μM). The levels of intracellular ROS and RNS were measured using DCF-DA and DHR123 assays, respectively, as described in Section 2. (**P<.01, as compared with cells treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 alone.)

JNK using Western blot analysis. Treatment of PC12 cells with hydrogen peroxide or SIN-1 induced phosphorylation of JNK, which was blocked by piceatannol (Fig. 6).

3.8. SP600125 inhibited hydrogen-peroxide- and peroxynitrite-induced PC12 cell death

To confirm the involvement of JNK activation in hydrogen-peroxide- and peroxynitrite-induced apoptosis, we examined whether SP600125 (an inhibitor of JNK) inhibited hydrogen-peroxide- and peroxynitrite-induced cell death. Pretreatment with 10 μM SP600125 attenuated hydrogen-peroxide- and peroxynitrite-induced cell death (Fig. 7).

4. Discussion

The oxidative stress and nitrosative stress caused by increased intracellular accumulation of ROS and RNS have been implicated in many pathophysiological processes, such as brain ischemia, inflammation and neurodegeneration, including AD [16]. Amyloid beta, which is considered to be responsible for the formation of senile plaques that accumulate in the brains of patients with AD, was found to induce apoptosis in cultured neurons via the generation of hydrogen peroxide [17] or peroxynitrite [18]. Thus, the accumulation of intracellular ROS and RNS is associated with the pathogenesis and/or progression of AD. There are multiple lines of evidence that a proper dietary regimen is important for maintaining cognitive functioning and preventing or delaying AD. One of the plausible ways to prevent the cellular injuries induced by oxidative or nitrosative stress is to augment or potentiate endogenous oxidative defense capacity through the dietary or pharmacological intake of antioxidants. Previous studies have shown that resveratrol exerts beneficial effects, including chemoprevention and cardioprotection. A previous study also demonstrated that resveratrol protects PC12 cells from amyloid-beta-induced apoptosis [19]. Piceatannol has been isolated — together with resveratrol — from grapes and wine and differs from resveratrol by possessing an additional aromatic hydroxyl group. In the present study, we found that piceatannol rescued both peroxynitrite- and hydrogen-peroxide-induced PC12 cell death.

Apoptosis is a gene-regulated mechanism of cell death that plays a pivotal role in physiological and pathological processes. Although the specific intracellular signaling pathways by which hydrogen peroxide and peroxynitrite trigger cell death are not yet well defined, recent studies have provided evidence for the involvement of nucleus condensation and PARP cleavage [12,19]. We found that piceatannol blocks hydrogen-peroxide- and peroxynitrite-induced nucleus condensation and PARP cleavage. The involvement

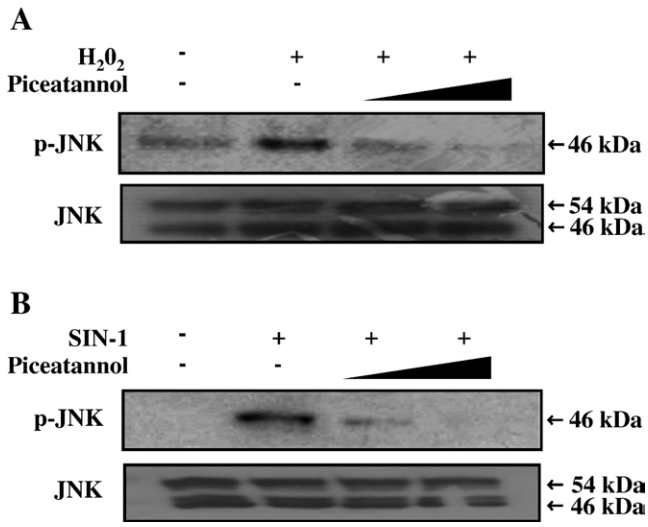

Fig. 6. Effects of piceatannol on phosphorylation of JNK induced by hydrogen peroxide (A) and SIN-1 (B). PC12 cells were incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 24 h in the presence or absence of piceatannol (10 and 20 μM). Western blot analyses were performed for phosphorylated and total JNK as described in Section 2. Data are representative of two independent experiments that gave similar results.

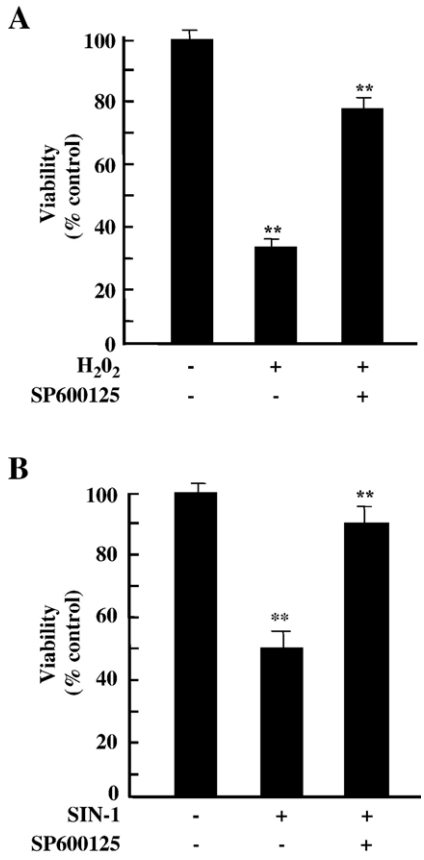

Fig. 7. Effects of SP600125 on the cytotoxic effects of hydrogen peroxide (A) and SIN-1 (B) in PC12 cells. PC12 cells were incubated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 for 24 h in the presence or absence of SP600125 (10 μM). The cell viability was determined using the MTT assay as described in Section 2. Data are mean±S.D. values for three independent experiments. (**P<.01, as compared with cells treated with 150 μM hydrogen peroxide or 1.5 mM SIN-1 alone.)

of mitochondria in apoptosis has been extensively investigated in numerous experimental systems. The members of the Bcl-2 family are also critical for the regulation of apoptosis [20]. They control the release of mitochondrial cytochrome *c* by modulating the permeability of the outer mitochondrial membrane and include antiapoptotic molecules (Bcl-2, Bcl-X_L, Bcl-W, Mcl-1 and A1). It has been reported that ectopic expression of *bcl-2* protects various cells from apoptosis induced by oxidative stress [13]. There are several lines of evidence that Bcl-2 acts via antioxidant pathways to prevent apoptosis. Bcl-X_L is a common antiapoptotic protein that plays a central role in cell survival. In the present study, exposure of PC12 cells to hydrogen peroxide or peroxynitrite resulted in the decreased Bcl-X_L expression, which was prevented by piceatannol treatment.

In general, apoptosis is driven from the activation of a family of cysteine proteases called caspases, which cleave a critical set of cellular proteins to initiate apoptotic cell death [15,21]. The family is expressed as proenzymes and activated by upstream stimuli. Among at least 14 known mammalian caspases, those involved with apoptosis can be further subdivided into the initiator caspases (caspase-2, -8, -9 and -10) and the effector caspases (caspase-3, -6 and -7). The two main pathways for activating caspases are the death-receptor- and mitochondria-mediated mechanisms. Caspase-3 is a key executioner caspase involved in neuronal apoptosis, and its activity is controlled by upstream regulators, such as caspase-8 and -9, which modulate the mitochondria- and death-receptor-dependent pathway, respectively. Previous studies have shown that ROS and RNS directly induce the activation of caspase-3 and -8 in PC12 cells [15,22,23]. We found that the activities of caspase-3 and -8 were increased in hydrogen-peroxide- and peroxynitrite-treated PC12 cells, indicating that the hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells is associated with the activation of caspase-3 and -8. Piceatannol reduced the hydrogen-peroxide- and peroxynitrite-induced activation of caspase-3 and -8 in PC12 cells.

Considerable attention has been paid to hydrogen peroxide and peroxynitrite because of their potential roles as secondary messengers in intracellular signaling networks. The specific intracellular signaling pathways by which hydrogen peroxide and peroxynitrite trigger cell death are not yet well defined. Previous studies have provided evidence for the involvement of JNK in the induction of apoptosis in several cell lines [12,19]. It is likely that certain phytochemicals inhibit oxidative-stress- or nitrosative-stress-mediated phosphorylation of JNK. Treatment of PC12 cells with hydrogen peroxide or peroxynitrite also led to phosphorylation of JNK, which was inhibited by piceatannol treatment. Furthermore, SP600125 (a JNK inhibitor) significantly inhibited hydrogen-peroxide- and peroxynitrite-induced PC12 cell death, revealing inactivation of the JNK pathway as a possible molecular mechanism for the protective effects of piceatannol against hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells. Many studies have suggested that antioxidants are able to reduce excess ROS and RNS and subsequently inhibit neuronal cell death. We found that piceatannol attenuates accumulation of hydrogen peroxide and peroxynitrite in PC12 cells. However, we did not exclude the possibility that piceatannol directly binds to protein kinases, including JNK, involved in oxidative neuronal cell death and inhibits their activity. In summary, these findings indicate that the protective effect of piceatannol against hydrogen-peroxide- and peroxynitrite-induced apoptosis of PC12 cells is associated with blocking activation of JNK and down-regulation of Bcl-X_L. Piceatannol might, therefore, be a useful chemopreventive agent for protecting against oxidative and nitrosative neuron cell death.

Acknowledgments

This work was supported by the Korea Institute of Science and Technology Evaluation and Planning for Functional Food Research and Development (no. 2007-01866) and the Korea Science and Engineering Foundation

(no. RO1-2007-000-11957-0), the Ministry of Science and Technology, Republic of Korea.

References

[1] Behl C. Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. Prog Neurobiol 1999;57:301–23.
[2] Selkoe DJ. Amyloid beta-protein and the genetics of Alzheimer's disease. J Biol Chem 1996;271:18295–8.
[3] Deigner HP, Haberkorn U, Kinscherf R. Apoptosis modulators in the therapy of neurodegenerative diseases. Expert Opin Investig Drugs 2000;9:747–64.
[4] Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239–47.
[5] Rhee SG. Redox signaling: hydrogen peroxide as intracellular messenger. Exp Mol Med 1999;31:53–9.
[6] Torreilles F, Salman-Tabcheh S, Guerin M, Torreilles J. Neurodegenerative disorders: the role of peroxynitrite. Brain Res Brain Res Rev 1999;30:153–63.
[7] Ono K, Hamaguchi T, Naiki H, Yamada M. Anti-amyloidogenic effects of antioxidants: implications for the prevention and therapeutics of Alzheimer's disease. Biochim Biophys Acta 2006;1762: 575–86.
[8] Lau FC, Shukitt-Hale B, Joseph JA. The beneficial effects of fruit polyphenols on brain aging. Neurobiol Aging 2005(Suppl 1): 128–32.
[9] Larrosa M, Tomas-Barberan FA, Espin JC. The grape and wine polyphenol piceatannol is a potent inducer of apoptosis in human SK-Mel-28 melanoma cells. Eur J Nutr 2004;43:275–84.
[10] Ferrigni NR, McLaughlin JL, Powell RG, Smith CR. Use of potato disc and brine shrimp bioassays to detect activity and isolate piceatannol as the antileukemic principle from the seeds of *Euphorbia lagascae*. J Nat Prod 1984;47:347–52.
[11] Wolter F, Clausnitzer A, Akoglu B, Stein J. Piceatannol, a natural analog of resveratrol, inhibits progression through the S phase of the cell cycle in colorectal cancer cell lines. J Nutr 2002;132: 298–302.
[12] Jang J-H, Surh Y-J. Beta-amyloid induces oxidative DNA damage and cell death through activation of c-Jun N terminal kinase. Ann N Y Acad Sci 2002;973:228–36.
[13] Jang J-H, Surh Y-J. Potentiation of cellular antioxidant capacity by Bcl-2: implications for its antiapoptotic function. Biochem Pharmacol 2003;66:1371–9.
[14] Jang J-H, Surh Y-J. Possible role of NF-kappaB in Bcl-X(L) protection against hydrogen peroxide-induced PC12 cell death. Redox Rep 2004;9:343–8.
[15] Wellington CL, Hayden MR. Caspases and neurodegeneration: on the cutting edge of new therapeutic approaches. Clin Genet 2000;57:1-10.
[16] Metodiewa D, Koska C. Reactive oxygen species and reactive nitrogen species: relevance to cyto(neuro)toxic events and neurologic disorders. An overview. Neurotox Res 2000;1:197–233.
[17] Tabner BJ, El-Agnaf OM, Turnbull S, German MJ, Paleologou KE, Hayashi Y, et al. Hydrogen peroxide is generated during the very early stages of aggregation of the amyloid peptides implicated in Alzheimer disease and familial British dementia. J Biol Chem 2005; 280:35789–92.
[18] Jang J-H, Aruoma OI, Jen LS, Chung HY, Surh Y-J. Ergothioneine rescues PC12 cells from beta-amyloid-induced apoptotic death. Free Radic Biol Med 2004;36:288–99.
[19] Jang J-H, Surh Y-J. Protective effect of resveratrol on beta-amyloid-induced oxidative PC12 cell death. Free Radic Biol Med 2003;34: 1100–10.
[20] Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science 1998;281:1322–6.
[21] Thornberry NA, Lazebnik Y. Caspases: enemies within. Science 1998; 281:1312–6.
[22] Marques CA, Keil U, Bonert A, Steiner B, Haass C, Muller WE, et al. Mechanisms caused by the Alzheimer's disease-linked Swedish amyloid precursor protein mutation. J Biol Chem 2003;278: 28294–302.
[23] Jung JY, Han CR, Jeong YJ, Kim HJ, Lim HS, Lee KH, et al. Epigallocatechin gallate inhibits nitric oxide-induced apoptosis in rat PC12 cells. Neurosci Lett 2007;411:222–7.