Mechanism of Inhibition of Human Cytochrome P450 1A1 and 1B1 by Piceatannol

Ah-Reum CHAE, Jae-Ho SHIM and Young-Jin CHUN*

College of Pharmacy, Chung-Ang University, Seoul 156-756, Republic of Korea

Abstract – The resveratrol analogue piceatannol (3,5,3',4'-tetrahydroxy-*trans*-stilbene) is a polyphenol present in grapes and wine and reported to have anti-carcinogenic activities. To investigate the mechanism of anticarcinogenic activities of piceatannol, the effects on CYP 1 enzymes were determined in *Escherichia coli* membranes co-expressing recombinant human CYP1A1, CYP1A2 or CYP1B1 with human NADPH-P450 reductase. Piceatannol showed a strong inhibition of CYP1A1 and CYP1B1 in a concentration-dependent manner, and IC_{50} of human CYP1A1 and CYP1B1 was 5.8 µM and 16.6 µM, respectively. However, piceatannol did not inhibit CYP1A2 activity in the concentration of up to 100 µM. Piceatannol exhibited 3-fold selectivity for CYP1B1 over CYP1A1. The mode of inhibition of piceatannol was non-competitive for CYP1A1 and CYP1B1. The result that piceatannol did not inhibit CYP1B1-mediated α-naphthoflavone (α-NF) metabolism suggests piceatannol may act as a non-competitive inhibitor as well. In human prostate carcinoma PC-3 cells, piceatannol induces apoptosis and prevents Akt-mediated signal pathway. Taken together, abilities of piceatannol to induce apoptotic cell death as well as CYP1 enzyme inhibition make this compound a useful tool for cancer chemoprevention.

Keywords : Cytochrome P450, Enzyme inhibition, Resveratrol, Piceatannol.

INTRODUCTION

Cytochrome P450 (CYP) enzymes are a superfamily of hemoprotein monooxygenases that catalyze the oxidation of a wide variety of both endogenous and xenobiotic compounds (Wrighton and Stevens, 1992; Kadlubar and Hammons, 1987; Guengerich and Shimada, 1991). The CYP enzymes are categorized into families and subfamilies by their sequence homology, and to date more than 1,200 CYP sequences were known. Among various human CYP enzymes, CYP1 enzymes such as CYP1A1 and CYP1B1 have continued to receive much attention because of their important roles for chemical carcinogenesis.

These enzymes are capable of catalyzing a number of oxidations of polycyclic aromatic hydrocarbons (PAHs). CYP1A1 is mainly expressed in extrahepatic tissues and induced by TCDD. The gene regulation of the CYP1A1 is known to be controlled by the Ah receptor-mediated signal pathway. CYP1B1 is characterized by its ability to metaboli-

cally activate 17β-estradiol as well as polycyclic aromatic hydrocarbons such as benzo[α]pyrene and 7,12-dimethyl-benz[α]anthracene (DMBA). CYP1B1 can also be induced by TCDD, similarly to the other members of CYP1 family. Because CYP1 enzymes play an important function in chemical carcinogenesis processes, this enzymes have been considered as a target enzymes for cancer chemoprevention. Previously, we reported for the first time that resveratrol (*trans*-3,4',5-trihydroxystilbene) acts as a selective inhibitor of CYP1A1 (Chun *et al*., 1999). Chang *et al*. (2000) also showed that resveratrol can inhibit the activity of CYP1B1 enzyme. Various resveratrol analogues were evaluated to find potently selective inhibitors of CYP1 enzymes. Rhapontigenin (3,3',5-trihydroxy-4'-methoxystilbene) was found to be a mechanism-based inactivator of CYP1A1 with an IC_{50} value of 0.4 mM (Chun *et al*., 2001b).

Strong selectivity of rhapontigenin for CYP1A1 over CYP1B1 (23-fold differences) indicated the structural differences of substrate binding sites of these enzymes. We also found 2,4,3',5'-tetramethoxystilbene, a methoxy derivative of oxyresveratrol, to be one of the most potently selective competitive inhibitor of P450 1B1 with an IC_{50} value of 6 nM (Chun *et al*., 2001a). 3,4,3',5'-Tetrahydroxystilbene known

*Corresponding author
Tel: +82-2-820-5616, Fax: +82-825-5616
E-mail: yjchun@cau.ac.kr

as piceatannol shares the structural similarity with resveratrol. The only difference between resveratrol and piceatannol is the presence of an extra hydroxyl group in one of the aromatic rings of piceatannol. Piceatannol was previously identified as antileukemic components (Ferrigni et al., 1984) and has been studied as a specific inhibitor of protein tyrosine kinase such as p72[Syk] (Geahlen and McLaughlin, 1989; Peters et al., 1996). It also inhibits various tyrosine kinases including the MAP kinases (Fleming et al., 1995) as well as STAT3 and STAT5 (Su and David, 2000). To determine whether piceatannol also acts as a specific inhibitor of CYP1 enzymes as resveratrol, in these studies, we determined the inhibition of CYP1 enzymes by piceatannol with the goal of identifying a new cancer chemopreventive agent to protect chemical carcinogenesis.

MATERIALS AND METHODS

Materials

7-Ethoxyresorufin, resorufin, DMSO, thiamine, IPTG, δ-aminolevulinic acid, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). Piceatannol was obtained from Calbiochem (Darmstadt, Germany). Bactopeptone, yeast extract, and bactoagar were from Difco Lab. (Detroit, MI). All other chemicals used were of the highest grade commercially available.

Recombinant Human CYPs

Coexpression (bicistronic) plasmids for human P450s (1A1, 1A2, or 1B1) and NADPH-P450 reductase were transformed into Escherichia coli (E. coli) DH5α (Parikh et al., 1997). A single ampicillin-resistant colony of transformed cells was selected and cultured overnight to saturation at 37°C in LB medium containing 100 μg/ml ampicillin. A 10-ml aliquot was used to inoculate each liter of Terrific Broth (TB) containing 0.2% bactopeptone (w/v), 100 μg/ml ampicillin, 1.0 mM thiamine, trace elements, 0.5 mM δ-aminolevulinic acid, and 1.0 mM IPTG. The cultures were grown at 30°C with shaking at 200 rpm for 48 h. Membrane fractions were prepared by differential centrifugation from bacteria and suspended in 10 mM Tris-HCl buffer (pH 7.4) containing 1.0 mM EDTA and 20% (v/v) glycerol (Guengerich et al., 1996).

EROD Enzyme Assays

EROD activity was determined to measure P450 1A1, 1A2, and 1B1 activities (Burke et al., 1985). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.4), 2 mg/ml bovine serum albumin, 2 μM ethoxyresorufin, and E. coli membranes (2 nM). The reaction mixtures were preincubated at 37°C for 3 min, and the reactions were initiated by adding 120 μM NADPH. Incubations were performed in a shaking water bath at 37°C for 20 min and were terminated by adding 1 ml of methanol. The formation of resorufin was determined fluorometrically with a Tecan GENios microplate reader (Tecan, Maennedorf, Switzerland) with excitation and emission wavelengths of 535 nm and 590 nm, respectively.

HPLC Analysis

Piceatannol (0.2 mM) was incubated in 0.1 M potassium phosphate buffer (pH 7.4), containing E. coli bicistronic membranes containing CYP1B1 (100 nM), NADPH-cytochrome P450 reductase, and NADPH (1 mM) in the presence of α-naphthoflavone (NF) in a final volume of 0.25 ml. Incubations were performed in a shaking water bath for 30 min at 37°C. The same experiments were performed without NADPH or with heat-inactivated membranes. The reaction was terminated by the addition of 1 ml of dichloromethane. After vigorously mixing for 5 min and then centrifugation for 10 min at 2000 g, the supernatant was transferred to a glass tube and dried with a nitrogen gas at 37°C. The residuals were dissolved in 50 μl of methanol. The reaction products were separated by HPLC on a 5-μm TSK-GEL ODS-100S column (4.6 mm × 250 mm) with 1% acetic acid/acetonitrile (40:60, v/v) as a mobile phase. The absorbance at 306 nm was measured using a Hitachi UV L-2400 detector (Hitachi, Tokyo, Japan).

Cell Viability Assay

Human prostate cancer PC-3 cells (1 × 10^4 cells/well) were plated onto 96-well plates and incubated at 37°C in a 5% CO_2 atmosphere. After incubation for the designated time, 10 μl of CCK-8 solution (Dojindo Molecular Technologies, Gaithersburg, MD) was added per well. After incubating at 37°C for 210 min, the absorbance at 450 nm was measured using a Tecan GENios microplate reader. The percentages of cells surviving from each group relative to control, defined as 100% survival, were calculated.

Apoptosis Assay

Cells were harvested and washed with the binding buffer (pH 7.4) containing 10 mM Hepes, 140 mM NaCl, and 2.5 mM $CaCl_2$. After resuspending in 0.2 ml of binding buffer, 5 μl of Annexin V-FITC (Clontech Laboratories, Palo Alto, CA) was added per well and cells were incubated for 10 min at room temperature. Cells stained with Annexin V-FITC were washed with the binding buffer. Apoptotic cells were analyzed using Olympus IX70 fluorescence microscope (Tokyo, Japan) with excitation and emission wavelengths of 488 nm and 518 nm, respectively.

Western Blot Analysis

Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Membranes were blocked in 5% (w/v) nonfat dried milk in Tris-buffered saline with 0.05 % tween 10 (TBST) overnight at 4°C. Membranes were then incubated for 1.5 h with primary antibodies at a 1:1000 dilution in TBST. After subsequent incubation with a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody, proteins were visualized by an ECL method.

Other Assays

The P450 content of the cells and membranes was determined by the spectral method of Omura and Sato (1964) using an extinction coefficient of 91 $mM^{-1}cm^{-1}$ with a Shimadzu UV-1650 UV/visible spectrophotometer (Shimadzu, Kyoto, Japan) at ambient temperature. Nitroblue tetrazolium (NBT) reduction was determined for the measurement of human NADPH-P450 reductase activity. The reaction mixture contained 0.1 M Tris-HCl buffer (pH 7.4), 100 μM of NBT, and 50 μg of *E. coli* membranes. After adding 40 μM NADPH, the absorbance at 530 nm was recorded for 5 min using Shimadzu UV-1650 UV/visible spectrophotometer

Data Analysis

Statistical analysis was performed by using one-way analysis of variance, followed by Dunnett's pairwise multiple comparison *t*-test with GraphPad Prism software (GraphPad Software Inc., San Diego, CA) when appropriate. The difference was considered statistically significant at $p < 0.05$.

RESULTS

Piceatannol inhibits human CYP1A1 and CYP1B1 in vitro

To examine the effects of piceatannol (Fig. 1) on the activities of CYP1A1, 1A2, and 1B1, the changes in EROD activities were determined with *E. coli* bicistronic membranes expressing human recombinant CYP1A1, 1A2, or 1B1 with human NADPH-P450 reductase (Fig. 2). Piceatannol showed a strong inhibition on CYP1A1 activity with IC_{50} values of 5.8 μM and to a lesser extent on CYP1B1 activity with IC_{50} values of 16.6 μM. However, the inhibition of CYP1A2-dependent activities was not strong up to 100 μM piceatannol. Thus, piceatannol exhibited 3-fold selectivity for CYP1B1 over CYP1A1. In human P450 systems, NADPH-P450 reductase can transfer electrons from NADPH to CYP enzymes and in
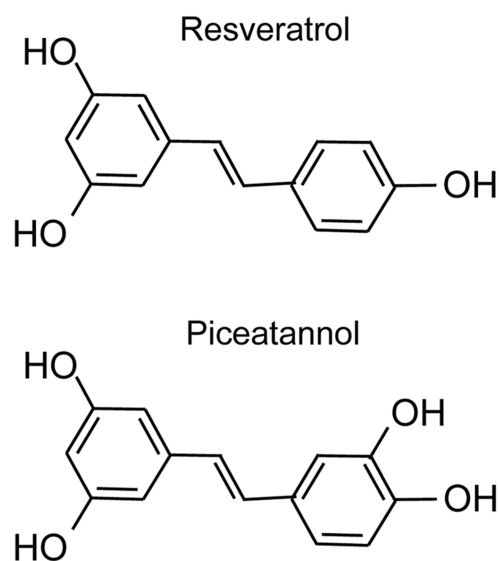

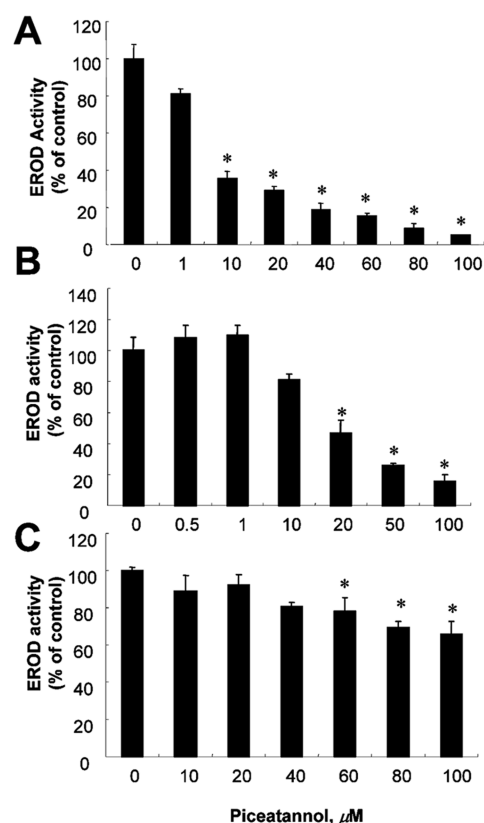**Fig. 1.** Structure of piceatannol and resveratrol.

Fig. 2. Effect of piceatannol on the human CYP1A1, 1A2, 1B1 activities in the bicistronic expression systems. Bacterial membranes coexpressing human CYPs and NADPH-P450 reductase were incubated with piceatannol for 20 min at 37°C in the presence of 120 μM NADPH. (A) CYP1A1, (B) CYP1B1, (C) CYP1A2. Each data point represents the mean ± S.D. of three experiments. *Significantly different from untreated control group (p < 0.05).
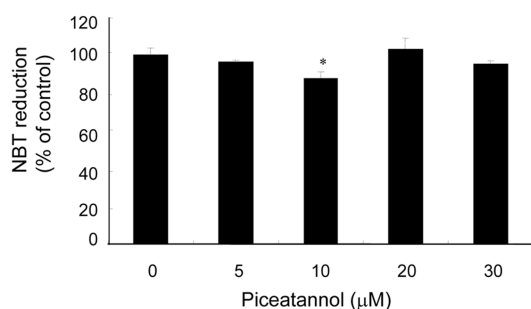
Fig. 3. Effect of piceatannol on NADPH-P450 reductase of *E. coli* membranes in which human CYP and NADPH-P450 reductase are coexpressed. Rates of NBT reduction were determined to measure NADPH-P450 reductase activity. Each data point represents the mean ± S.D. of three experiments. [*]Significantly different from untreated control group (p < 0.05).

some cases the inhibition of CYP activity by chemicals is mediated via blocking of electron transfer by the inhibition of NADPH-P450 reductase activity. To determine whether piceatannol can inhibit human NADPH-P450 reductase, NBT reduction was measured as a surrogate
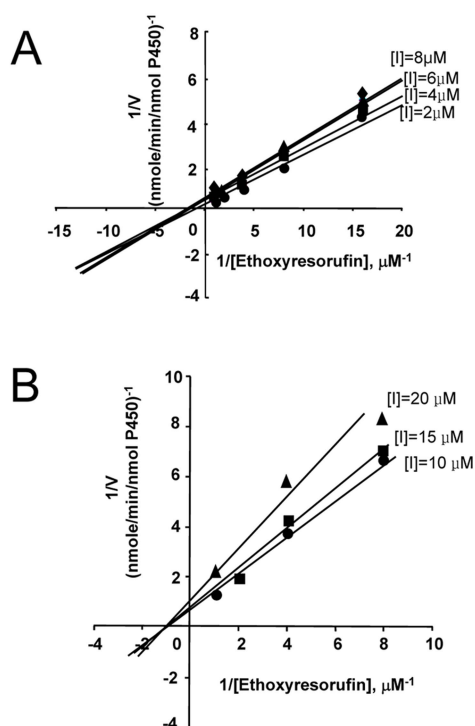
assay for NADPH-P450 reductase-dependent reduction reaction. Figure 3 showed that piceatannol did not inhibit NADPH-P450 reductase activity up to 30 μM.

Mechanism of Enzyme Inhibition by Piceatannol

To elucidate the mechanism of inhibition of CYP1 enzymes by piceatannol, kinetic studies were performed using coexpressed *E. coli* membranes. Analysis of the mode of inhibition indicated noncompetitive inhibition for CYP1A1 and CYP1B1 with K_i of 1.7 and 4.7 μM, respectively (Fig. 4). We had also determined whether piceatannol decreases metabolism of α-NF by CYP1B1. Because α-NF is known as a substrate as well as a competitive inhibitor of CYP1B1-mediated EROD activity, if piceatannol works as a real noncompetitive inhibitor of CYP1 enzymes, the metabolism of α-naphthoflavone (NF) by CYP1B1 may not be blocked. Fig. 5 showed that piceatannol could not block the CYP1B1-mediated generation of an α-NF metabolite (M).
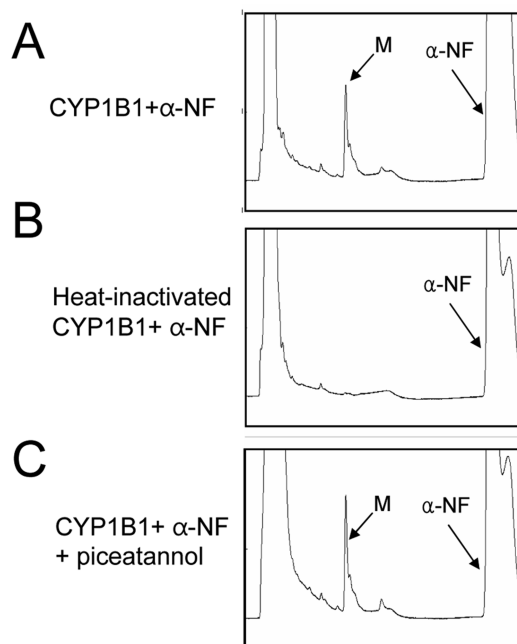

Fig. 4. Kinetic analysis of CYP1A1 and CYP1B1 inhibition by piceatannol. EROD activity was determined with *E.coli* membrane coexpressed human CYPs and NADPH-P450 reductase in the presence of piceatannol. Lineweaver-Burk plot was plotted and the kinetic parameters were calculated using nonlinear regression methods. (A) CYP1A1. (B) CYP1B1. Each data point represents the mean ± S.D. of three experiments.

Fig. 5. Effect of piceatannol on α-NF metabolism by human CYP1B1. Piceatannol (0.2 mM) was incubated with bacterial membranes expressing human CYP1B1 in 0.1 M potassium phosphate buffer (pH 7.4) in the presence of 1 mM NADPH and 0.2 mM α-NF for 30 min at 37°C. The metabolite generated was analyzed using HPLC (C_{18} reverse phase column, 4.6 mm × 250 mm). The retention times of α-NF and its metabolite (M) were 26.1 and 10.7 min, respectively. (A) Active CYP1B1 was incubated with a-NF in the presence of NADPH. (B) Heat-inactivated CYP1B1 was incubated with α-NF in the presence of NADPH. (C) Active CYP1B1 was incubated with α-NF in the presence of NADPH and piceatannol.
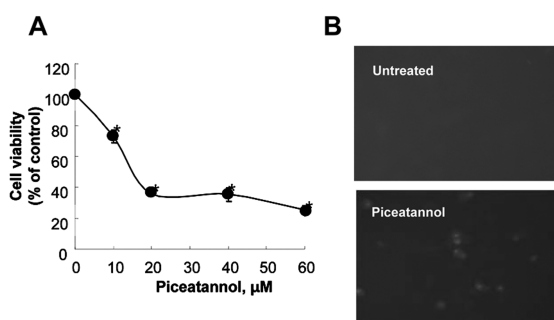
A

B

Fig. 6. Induction of apoptosis by piceatannol in PC-3 cells. (A) Cells (1×10^4/well) were incubated with the indicated concentration of piceatannol for 24 h. The percentage of cells surviving from each groups relative to controls were determined by CCK-8 assay. Results are the mean ± S.D. of three separate experiments. *Significantly different from DMSO-treated control group ($p < 0.05$). (B) Cells (1×10^5/ml) were incubated with piceatannol (60 μM) for 24 h. After staining cells with Annexin V-FITC, positive cells were analyzed by fluorescence microscopy.

Piceatannol Induces Apoptosis Through Akt Inactivation

To determine the potential of piceatannol as an adjuvant agent for cancer chemoprevention, the cytotoxic effects of piceatannol in human prostate cancer PC-3 cells were determined. Piceatannol showed a concentration-dependent inhibition of cellular proliferation in the cultured cells for 24 h (Fig. 6A). To elucidate the ability of TMS to induce apoptotic cell death in PC-3 cells, Annexin V-positive cells were determined using fluorescence microscopy. Fig. 6B showed that treatment with piceatannol (60 μM) for 24 h caused an appreciable increase in Annexin V-positive cells, which is indication of apoptotic cell death. To explore the mechanism responsible for the apoptosis, we examined the possibility that piceatannol might attenuate the anti-apoptotic activity of Akt. PC-3 cells were exposed up to 80 μM piceatannol for 24 h and Akt and phospho-Akt levels were examined by Western blot analysis. Fig. 7
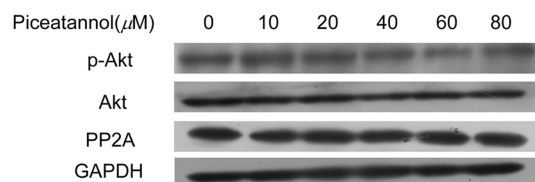

Fig. 7. Inactivation of Akt by piceatannol. Cells (1×10^5/ml) were incubated with piceatannol (0-80 μM) for 24 h. Total cellular sonicates were prepared and used for Western blot analyses with antibodies against phospho-Akt, Akt, PP2A, or GAPDH.

showed that piceatannol significantly decreased the level of phosphor-Akt although Akt and protein phosphatase 2A (PP2A) levels remained unaltered.

DISCUSSION

The present study was designed to examine how piceatannol, a naturally occurring analog of resveratrol (*trans*-3,5,4'-trihydroxystilbene; Fig. 1) exerts its effects as a chemopreventive agent. One important function is as a specific inhibitor of CYP1 enzymes. Previously we have extensively studied that resveratrol and various analogues are specific inhibitors of CYP1 enzymes (Chun *et al.*, 1999; Chun *et al.*, 2001a; Chun *et al.*, 2001b; Chun and Kim, 2003; Lee *et al.*, 2004). Resveratrol exhibited inhibition of CYP1A1 activity with IC_{50} of 23 μM for ethoxyresorufin O-deethylation and 11 μM for methoxyresorufin O-demethylation. Rhapontigenin (3,3',5-trihydroxy-4'-methoxystilbene) showed a potent and selective inhibition of CYP1A1 with IC_{50} value of 0.4 μM and 3,4'-dimethoxy-5-hydroxystilbene showed inhibition of EROD activity of both CYP1A1 and CYP1B1 with an IC_{50} value of 0.1 μM (Chun *et al.*, 2001b). 2,4,3',5'-Tetramethoxystilbene (2,4,3',5'-TMS), a methoxy derivative of oxyresveratrol, is suggested as the one of the most potently selective competitive inhibitor of CYP1B1 with IC_{50} value of 6 nM (Chun *et al.*, 2001a). Our present data showed that piceatannol also inhibits human CYP1A1 and 1B1 with IC_{50} values of 5.8 μM and 16.6 μM. K_i values for noncompetitive inhibition were 1.7 and 4.7 μM for CYP1A1 and CYP1B1, respectively. Although piceatannol might be a weaker inhibitor of CYP1A1 or CYP1B1 compared to a synthetic potently selective inhibitor such as 2,4,3',5'-TMS, it shows stronger inhibition than resveratrol. Moreover, the mode of inhibition may be noncompetitive rather than competitive. It is quite interesting because resveratrol is known to act as a competitive inhibitor for CYP1A1 or CYP1B1.

The structural difference between resveratrol and piceatannol is an additional hydroxyl group at 4-position on phenyl ring 1. Although the role of this hydroxyl group on inhibition mechanism needs to be determined, it may play an important role in binding piceatannol on the uncharacterized regulatory sites and cause a significant change in the CYP structure. The other function of piceatannol as a chemopreventive agent would be antiproliferative effect in cancer cells. Since resveratrol's effect as a inducer of apoptosis have been studied (Hsieh *et al.*, 1999; Surh *et al.*, 1999; Tsan *et al.*, 2000), increasing interest has been paid to *trans*-stilbene compounds. Previous our studies showed that 3,4'-dimethoxy-5-hydroxystilbene induces apoptosis in

HL-60 cells (Lee *et al.*, 2002). 2,4,3',5'-TMS also exerted to induce apoptosis in human cancer cells (Chun *et al.*, 2005). Our present results also showed that piceatannol induces apoptosis through the inactivation of Akt. The PI3 kinase/Akt pathway is important to regulate diverse cellular processes including cell survival and proliferation (Kennedy *et al.*, 1997; Zhou *et al.*, 2000). Hyperactivation of Akt is a common event in many human cancers (Benistant *et al.*, 2000; Altomare and Testa, 2005). Activated Akt inhibits apoptosis through modulation of apoptosis regulatory proteins such as Bax, Bad, or IAPs (Gardai *et al.*, 2004; Johnson *et al.*, 2004; Tsuruta *et al.*, 2002; Zha *et al.*, 1996).

It has been proposed that suppression of Akt action may result in the onset of apoptosis, due to the dissociation of Bad from 14-3-3 proteins and translocation to the mitochondria, where Bad interacts with Bcl-2 family proteins (Zha *et al.*, 1996; Gross *et al.*, 1999). Recently, we showed that a hydrophilic stilbene analog (1-(2-{3-[2-(2,4-dimethoxy-phenyl)-vinyl]-5-methoxy-phenoxy}ethyl)-1H-imidazole) induces apoptosis through the inactivation of Akt and activation of p38 MAPK, resulting in activation of mitochondrial death signaling pathway (So *et al.*, 2008). Resveratrol is also known to inactivate Akt in various cancer cells (Aziz *et al.*, 2006; Li *et al.*, 2006; Cecchinato *et al.*, 2007). Thus, the detailed mechanism by which piceatannol exert antiproliferative effect in cancer cells warrant further investigation.

In summary, the present study proposes that piceatannol may be valuable for cancer chemoprevention because this compound contains a potential to inhibit carcinogen-activating enzymes such as CYP1A1 and CYP1B1 as well as to induce apoptotic cell death in human cancer cells.

ACKNOWLEDGEMENT

REFERENCES

Altomare, D. A. and Testa, J. R. (2005). Perturbations of the AKT signaling pathway in human cancer. *Oncogene* **24,** 7455-7464.

Aziz, M. H., Nihal, M., Fu, V. X., Jarrard, D. F. and Ahmad, N. (2006). Resveratrol-caused apoptosis of human prostate carcinoma LNCaP cells is mediated via modulation of phosphatidylinositol 3'-kinase/Akt pathway and Bcl-2 family proteins. *Mol. Cancer Ther.* **5**, 1335-1341.

Benistant, C., Chapuis, H. and Roche, S. (2000). A specific function for phosphatidylinositol 3-kinase alpha (p85 alpha-p110 alpha) in cell survival and for phosphatidylinositol 3-kinase beta (p85 α-p110 α) in de novo DNA synthesis of human colon carcinoma cells. *Oncogene* **19**, 5083-5090.

Burke, M. D., Thompson, S., Elcombe, C. R., Halpert, J., Haaparanta, T. and Mayer, R. T. (1985). Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P-450. *Biochem. Pharmacol.* **34**, 3337-3345.

Cecchinato, V., Chiaramonte, R., Nizzardo, M., Cristofaro, B., Basile, A., Sherbet, G. V. and Comi, P. (2007). Resveratrol-induced apoptosis in human T-cell acute lymphoblastic leukaemia MOLT-4 cells. *Biochem. Pharmacol.* **74**, 1568-1574.

Chang, T. K., Lee, W. B. and Ko, H. H. (2000). Trans-resveratrol modulates the catalytic activity and mRNA expression of the procarcinogen-activating human cytochrome P450 1B1. *Can. J. Physiol. Pharmacol.* **78**, 874-881.

Chun, Y. J., Kim, M. Y. and Guengerich, F. P. (1999). Resveratrol is a selective human cytochrome P450 1A1 inhibitor. *Biochem. Biophys. Res. Commun.* **262**, 20-24.

Chun, Y. J. and Kim, S. (2003). Discovery of cytochrome P450 1B1 inhibitors as new anti-cancer agents. *Med. Res. Rev.* **23**, 657-668.

Chun, Y. J., Kim, S., Kim, D., Lee, S. K. and Guengerich, F. P. (2001a). A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. *Cancer Res.* **61**, 8164-8170.

Chun, Y. J., Lee, S.K. and Kim, M. Y. (2005). Modulation of human cytochrome P450 1B1 expression by 2,4,3',5'-tetramethoxystilbene. *Drug Metab. Dispos.* **33**, 1771-1776.

Chun, Y. J., Ryu, S. Y., Jeong, T. C. and Kim, M. Y. (2001b). Mechanism based inhibition of human cytochrome P450 1A1 by rhapontigenin. *Drug Metab. Dispos.* **29**, 389-393.

Ferrigni, N. R., McLaughlin, J. L., Powell, R. G. and Smith, C. R. (1984). Isolation of piceatannol as the antileukemic principle from the seeds of euphorbia lagascae. *J. Nat. Prod.* **47**, 347-352.

Fleming, I., Fisslthaler, B. and Busse, R. (1995). Calcium signaling in endothelial cells involves activation of tyrosine kinases and leads to activation of MAP kinases. *Circ. Res.* **76**, 522-529.

Gardai, S. J., Hildeman, D. A., Frankel, S. K., Whitlock, B. B., Frasch, S. C., Borregaard, N., Marrack, P., Bratton, D. L. and Henson, P. M. (2004). Phosphorylation of Bax Ser184 by Akt regulates its activity and apoptosis in neutrophils. *J. Biol. Chem.* **279**, 21085-21095.

Geahlen, R. L. and McLaughlin, J. L. (1989). Piceatannol (3,4,3',5'-tetrahydroxy-trans-stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. *Biochem. Biophys. Res. Commun.* **165**, 241-245.

Gross, A., McDonnell, J. M. and Korsmeyer, S. J. (1999). Bcl-2 family members and the mitochondria in apoptosis. *Genes Dev.* **13**, 1899-1911.

Guengerich, F. P., Martin, M. V., Guo, Z. and Chun, Y. J. (1996). Purification of functional recombinant P450s from bacteria. *Methods Enzymol.* **272**, 35-44.

Guengerich, F. P. and Shimada, T. (1991). Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.* **4**,381-407.

Hsieh, T., Juan, G., Darzynkiewicz, Z. and Wu, J. M. (1999). Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21(WAF1/CIP1), and suppresses cultured

bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2. *Cancer Res.* **59**, 2596-2601.

Johnson, N. C., Dan, H. C., Cheng, J. Q. and Kruk, P. A. (2004). BRCA1 185delAG mutation inhibits Akt-dependent, IAP-mediated caspase 3 inactivation in human ovarian surface epithelial cells. *Exp. Cell Res.* **298**, 9-16.

Kadlubar, F. F. and Hammons, G. J. (1987). The role of cytochrome P-450 in the metabolism of chemical carcinogens. In: Mammalian cytochromes P-450. Boca Raton: CRC Press; 1987. pp 81-130.

Kennedy, S. G., Wagner, A. J., Conzen, S. D., Jordan, J., Bellacosa, A., Tsichlis, P. N. and Hay, N. (1997). The PI3-kinase/Akt signaling pathway delivers an anti-apoptotic signal. *Genes Dev.* **11**, 701-713.

Lee, S. K., Kim, Y., Kim, M. Y., Chun, Y. J. and Kim, S. (2004). Potent inhibition of recombinant human cytochrome P-450 1A1 by pentamethoxystilbene. *J. Toxicol. Environ. Health Part A* **67,** 1987-2000.

Lee, S. H., Ryu, S. Y., Kim, H. B., Kim, M. Y. and Chun, Y. J. (2002). Induction of apoptosis by 3,4'-dimethoxy-5-hydroxystilbene in human promyeloid leukemic HL-60 cells. *Planta Med.* **68**, 123-127.

Li, Y., Liu, J. Y., Liu, X. P., Xing, K. F., Wang Y., Li, F. Y. and Yao, L. (2006). Resveratrol-induced cell inhibition of growth and apoptosis in MCF7 human breast cancer cells are associated with modulation of phosphorylated Akt and caspase-9. *Appl. Biochem. Biotechnol.* **135,**181-192.

Omura, T. and Sato, R. (1964). The carbon-monoxide binding pigment of liver microsomes. *J. Biol. Chem.* **239,** 2370-2378.

Peters, J. D., Furlong, M. T., Asai, D. J., Harrison, M. L. and Geahlen, R. L. (1996). Syk, activated by cross-linking the B-cell antigen receptor, localises to the cytosol where it interacts with and phosphorylates a-tubulin on tyrosine. *J. Biol. Chem.* **271**, 4755-4762.

Parikh, A., Gillam, E. M. and Guengerich, F. P. (1997). Drug metabolism by Escherichia coli expressing human cytochromes P450. *Nat. Biotechnol.* **15**, 784-788.

So, K. S., Oh, J. E., Han, J. H., Jung, H. K., Lee, Y. S., Kim, S. H., Chun, Y. J. and Kim, M. Y. (2008). Induction of apoptosis by a stilbene analog involves Bax translocation regulated by p38 MAPK and Akt. *Arch. Pharm. Res.* **31**, 438-444.

Su., L. and David, M. (2000). Distinct mechanisms of STAT phosphorylation via the interferon-a/b receptor, selective inhibition of STAT3 and STAT5 by piceatannol. *J. Biol. Chem.* **275**, 12661-12666.

Surh, Y. J., Hurh, Y. J., Kang, J. Y., Lee, E., Kong, G. and Lee, S. J. (1999). Resveratrol, an antioxidant present in red wine, induces apoptosis in human promyelocytic leukemia (HL-60) cells. *Cancer Lett.* **140**, 1-10.

Tsan, M. F., White, J. E., Maheshwari, J. G., Bremner, T. A. and Sacco, J. (2000). Resveratrol induces Fas signalling-independent apoptosis in THP-1 human monocytic leukemia cells. *Brit. J. Haematol.* **109**, 405-412.

Tsuruta, F., Masuyama, N. and Gotoh Y. (2002). The phosphatidylinositol 3-kinase (PI3K)-Akt pathway suppresses Bax translocation to mitochondria. *J. Biol. Chem.* **277**, 14040-14047.

Wrighton, S. A. and Stevens, J. C. (1992). The human hepatic cytochrome P450 involved in drug metabolism. *Crit. Rev. Toxicol.* **22**,1-21.

Zha, J., Harada, H., Yang, E., Jockel, J. and Korsmeyer, S. J. (1996). Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3- not Bcl-XL. *Cell* **87**, 619-628.

Zhou, H., Li, X. M., Meinkoth, J. and Pittman, R. N. (2000). Akt regulates cell survival and apoptosis at a postmitochondrial level. *J. Cell. Biol.* **151**, 483-494.