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Activation of PKC-βII is Required for Vitamin E-Succinate-Induced Apoptosis of U937 Cells

Song-Ja Kim, Jae-Han Park, Sun-Ryung Lee, Ok-Sun Bang*, and Shin-Sung Kang

Department of Biology, College of Natural Sciences, Kyungpook National University, Taegu 702-701, Korea

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Vitamin E-succinate (VES) treatment of U937 human monoblasts induced cells to undergo apoptosis. After 96 h of VES treatment at 10 μg/ml, more than 80% of cells appeared apoptotic. Evidence for apoptosis by VES was based on propidium iodide staining for detection of chromatin condensational fragmentation and electrophoretic DNA ladder formation. Western blot analyses showed a transient increase in Fas and p21 protein levels up to 48 h after the VES treatment. Protein expression and activity of CDK1 and lamin B degradation were remarkably induced by VES, following the cleavage of caspase-3 after 48 h. The VES-induced apoptosis was found to involve activation of PKC as shown by increases in membrane translocation of PKCβII and PKC activity. Pretreatment of GF109203X (PKC inhibitor) prior to VES treatment almost completely inhibited the induction of apoptosis as assessed by blockage of VES-induced caspase-3 activity and DNA fragmentation. However, GF109203X had no effect on the VES-induced nitric oxide synthesis, which was required for monocytic differentiation in our previous report (*J Cell Sci* 111, 435, 1998). Taken together, our data suggest that induction of apoptosis by VES in U937 cells occurs through activation of PKC-βII resulting in the activation of caspase-3 cascade and is independent of nitric oxide.

Vitamin E-succinate (VES; RRR-alpha-tocopheryl succinate) has recently been reported to induce apoptosis in prostate cancer, breast carcinoma, lymphoma, and leukemia cells (Qian et al., 1997; Sokoloski et al., 1997; Zhao et al., 1997; Turley et al., 1997; Yu et al., 1998; Neuzil et al., 1999; Yu et al., 1999; Israeal et al., 2000). Little is known about the molecular mechanism of VES action in apoptosis. Our previous studies showed that VES induced dramatic inhibition of cell growth and monocytic differentiation of U937 cells that was mediated by nitric oxide (NO) (Kim et al., 1998). It can be suggested that the VES-induced cell growth inhibition may be caused by apoptotic cell death.

It has been expected that apoptosis is mediated by signal transduction pathways involving specific protein kinases. The phospholipid-dependent protein kinase C (PKC) family of isozymes has a central role in the transduction of extracellular signals and has been implicated in tumor promotion (Hug and Sarre., 1993). PKC can act not only as a negative regulator of cell death but also a potentiator of the apoptotic effect of other inducers depending upon cell system and apoptosis inducer (Schutze et al., 1994; Mansat et al., 1997; Porn-Ares et al., 1997).

The purpose of this study was to explore the underlying mechanism of VES-induced apoptosis on the basis of biochemical events. We show that the VES-induced apoptosis in U937 cell involves activation of PKC-βII and is unrelated to nitric oxide.

*To whom correspondence should be addressed.
Tel: 82-53-950-5349, Fax: 82-53-953-3066
E-mail: bos4444@chollian.net

Materials and Methods

Cell culture and treatments

U937 cell was obtained from the American Type Culture Collection and was maintained in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 100 IU/ml penicillin, and 50 μg/ml streptomycin (Gibco BRL) at 37℃ in a humidified 5% CO_2 incubator. Prior to treatments, cells were made quiescent by incubation in serum free-DMEM for 1 h. Cells were then treated with 10 μg/ml of VES or vehicle (0.1% ethanol and 5 μg/ml succinic acid). Chemicals used were 100 nM GF-109203X (Calbiochem) and 2 mM N-G-monomethyl-L-arginine (L-NMMA, Sigma).

Confocal microscopy

Control and VES-treated cells were washed three times with PBS, fixed with cold methanol:acetone (1:1), and stained with propidium iodide (PI, 50 μg/ml; Molecular Probes) containing RNase (40 μg/ml; Boehringer Mannheim). Morphological changes were analyzed by laser scanning confocal microscopy using a Carl Zeiss 410 confocal microscope (Carl Zeiss; Axiovert 135; Lens 3 x 40/0.75; laser line=488, 543).

DNA fragmentation assay

The pattern of DNA cleavage was analyzed by agarose gel electrophoresis as described (Maciejewski et al., 1995). Briefly, genomic DNA was purified by WizardTM Genomic DNA purification kit (Promega). After ethanol precipitation, 5 μg sample was loaded in each lane and was subjected to electrophoresis on a 1.4% agarosegel at 50 V for 3 h. DNA was stained with ethidium bromide.

Western blot analysis

Cells were lysed in 10 mM Tris-HCl, pH 7.4, containing 1% of sodium dodecyl sulfate (SDS), 10 μg/ml leupeptin, 2 mM PMSF, 2 μg/ml aprotinin, 10 μg/ml pepstain A, and 2 mM phenanthroline. Cell lysates were centrifuged for 10 min at 12,000 x g and supernatants were analyzed for protein concentration using a BCA protein assay kit (Pierce). Equal amounts of proteins were separated on 7.5 or 10% polyacrylamide gels containing 1% SDS and were transferred to nitrocellulose membrane. The membranes were probed with antibodies against PKC-α, βI, βII, γ, δ, ε, λ/ι and ζ isoforms, and CDK1 (Santa Cruz), and visualized by using ECL system (Amersham). The other proteins were detected by using antibodies against Fas (UBI), p21 (Transduction Lab), caspase-3 and lamin B (CalBiochem). Separation of cytosolic and membrane fractions of PKC isoforms was performed as described previously (Chang et al, 1998).

In vitro kinase assay

Two hundred μg of protein from total cellular lysates was incubated with anti-CDK1 antibody for 4 h at 4℃. Immnuocomplexes were collected with 15 μl of protein A-Sepharose slurry (50% v/v, Pharmacia), and washed three times with lysis buffer. Immunoprecipitates were washed twice with kinase buffer (20 mM Tris-HCl, pH 7.5, I mM dithiothreitol, 10 mM $MnCl_2$, and 10 mM $MgCl_2$), and incubated with 30 μl of kinase buffer containing 5 μCi of [γ-^{32}P] ATP (Amersham), 3 μg of histone H1 (CalBiochem) as a substrate, and 30 μM cold ATP for 30 min at 30℃. The reaction was stopped by adding 4 x SDS sample buffer followed by boiling for 5 min. Samples were then subjected to 12% SDS-PAGE followed by autoradiography.

FACS analysis

Cells were trypsinized and collected by centrifugation at 1,000 x g for 10 min. Collected cells were washed twice and resuspended into 1-2 x 10^6/ml with PBS. For fixation, equal volume of 80% ethanol was added dropwise and the cell suspension was kept overnight at 4℃. Cells were washed once with PBS, and then stained with PI solution containing 50 mg/ml PI, 0.1% Triton X-100, 0.1 mM EDTA and 50 mg/ml RNase for 60 min at room temperature. Stained DNA was analyzed by a flow cytometer (FACS Calibur; Beckton Dickinson). To detect PI, cells were excited at 488 nm, and emissions were observed at 585 nm.

Measurement of PKC activity

Quantification of PKC activity was carried out using SignaTECT PKC assay system (Promega) according to the manufacturer's instruction. Briefly, cells (5 x 10^6 cells) were washed in PBS and suspended in 0.5 ml of cold extraction buffer (50 mM Tris-HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, 0.05% Triton X-100, 10 mM 2-mercaptoethanol, and 1 μg/ml leupeptin, 1 μg/ml aprotinin) with 0.5 ml of 100 mM PMSF. The mixture was then homogenized using a Dounce homogenizer for 5 min. The homogenate was centrifuged for 5 min at 14,000 x g and the supernatant was saved. Reaction mixture was prepared consisting of 5 μl of PKC coactivation 5X buffer (1.25 mM EGTA, 2 mM $CaCl_2$, and 0.5 mg/ml BSA), 5 μl of PKC activation 5X buffer (100 mM Tris-HCl, pH 7.5, 1.6 mg/ml phosphatidylserine, 0.16 mg/ml diacylglycerol, and 50 mM $MgCl_2$), 5 μl of PKC biotinylated peptide substrate and 5 μl of ATP mixture (5 μl of 0.5 mM ATP and 0.05 μl of [γ-^{32}P] ATP (3,000 Ci/mmol)). The reaction mixture was preincubated for 5 min at 30℃, to which 5 μl of sample (20 μg/μl of protein in sample buffer) was added, followed by further incubation for 5 min. The reaction was stopped by adding 12.5 μl of 7.5 M guanidine-HCl. Ten μl of each reacted mixture was dropped onto SAM^{2TM} membrane. After serial washing, the membranes were dried for 1 h and radioactivity was determined with liquid scintillation counter (Wallac Model). The PKC activity was calculated as pmol of ATP/min/μg of protein.

Measurement of caspase activity

Caspase activity was analyzed using caspase fluorometric substrate (Ac-DEVD-aminomethylcoumarin; UBI) as described by Nicholson et al. (1995). Briefly, treated cells were washed in PBS and then lysed in modified RIPA buffer (50 mM Tris-HCl, pH 7.4 buffer containing 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EGTA). Fifty μg of lysate proteins were mixed with 1 ml of RIPA buffer and 7 μl of substrate, and incubated at 37℃ for 5 min. Fluorescence was then monitored with an excitation wavelength of 380 nm and an emission wavelength of 460 nm using fluorescence spectrophotometer (Kontron Model SFM25). Fluorescence of the substrate alone was subtracted from all measurements.

Measurement of nitrite

NO synthesis in cell cultures was measured by a microplate assay as previously described (Kim et al., 1998). One hundred μl of conditioned medium was collected and incubated with an equal volume of the Griess reagent [1% sulfanilamide, 0.1% N-(1-naphthyl)-ethylene-diaminedihydro-chloride, 2.5% H_3PO_4] at room

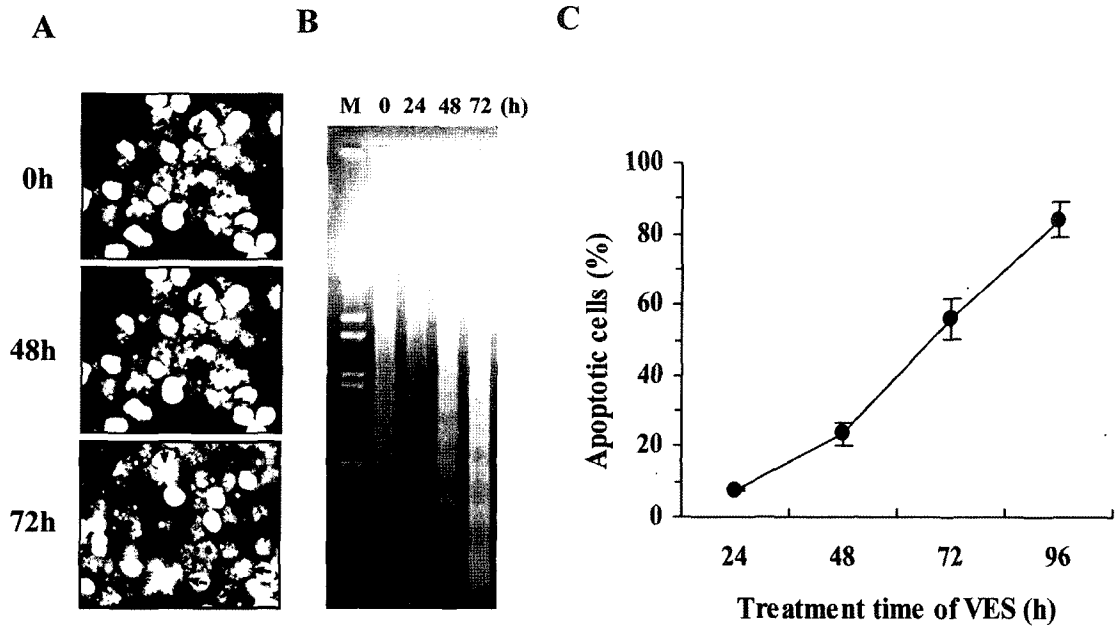

Fig. 1. Induction of apoptosis by VES in U937 cells. Cells were treated with 10 μg/ml VES for indicated periods. A, Confocal microscopy of cells stained with PI. Arrows indicates apoptotic cells with highly condensed chromatin and apoptotic bodies. B, DNA fragmentation analysis. Equal amounts of DNA (10 μg) were analyzed by 1.4% agaose gel electrophoresis and DNA laddering was visualized by staining gels with ethidium bromide. BstEII-digested λDNA was used as a molecular size marker (M). C, Quantification of apoptotic cells. Percentages of apoptotic cells were determined by counting more than 200 cells on PI stained cytospin slides.

temperature for 10 min. The absorbance at 540 nm was determined in a Titertek Multiskan (Flow Lab). NO_2^- was determined using sodium nitrite as standard.

Results

VES induces apoptosis in U937 cells

When U937 cells were treated with VES, a large number of cells began to display morphological changes characteristic of apoptotic cell death such as chromatin condensation and nuclear fragmentation after 48 h of culture. More than 80% of the cells appeared apoptotic at 96 h (Fig. 1A & C). Also oligonucleosomal length DNA fragmentation, a hallmark of apoptosis, appeared in the VES-treated cells after 48 h (Fig. 1B). The results indicated that VES induced apoptosis in U937 cells.

Expression of the apoptosis-related proteins such as Fas and p21 in the VES-treated U937 cells were analyzed by Western blotting. Their expression was dramatically increased up to 48 h followed by a decrease (Fig. 2A). The expression and activity of CDK1 showed similar pattern as those of Fas and p21 (Fig. 2B). The unscheduled elevation of CDK1 activity is reported to be related to apoptosis accompanied by diminished levels of lamin B dependent on caspase cleavage (Shimizu et al., 1995; 1998). Thus, we examined expression of caspase-3 and lamin B by Western blot analysis. The cleavage of lamin B appeared after 48 h in the VES-treated culture (Fig. 2C). The cleavage and activity of caspase-3 were maximal at 72 h after the VES treatment (Fig. 2C and Fig. 4A).

VES induces activation of PKC-βII

Recently, it has been reported that activation of PKC is related with apoptosis via a distinct signaling pathways (Shao et al., 1997; Cross et al., 2000, Frasch et al., 2000). Human promonocytic leukemia U937 cells expressed multiple PKC isoforms including α, βI, βII, δ, ε and ζ except PKC-γ and λ/ι. The expression level of PKC isoforms was not changed by the VES treatment (data not shown). To assess whether VES modulated PKC activity, first we performed Western blot analysis to visualize translocation of PKC from cytosol to membrane. Among the expressed PKC isoforms, only PKC-βII was translocated to membrane in response to the VES treatment which was maximal at 48-72 h (Fig. 3A). Most of the expressed PKC-isoforms except PKC-βII was detected in the cytosolic fraction without significant changes during the VES-induced apoptosis progression. We then tested the effect of VES on PKC activity. PKC preparation from the cell lysate was incubated in the presence of phosphatidylserine and diacylglycerol. VES remarkably increased PKC activity after 48-72 h (Fig. 3B). This is consistent with the translocation pattern of PKC-βII.

Involvement of activation of PKC-βII in VES-induced apoptosis in U937 cells

To examine the relationship between the activation of

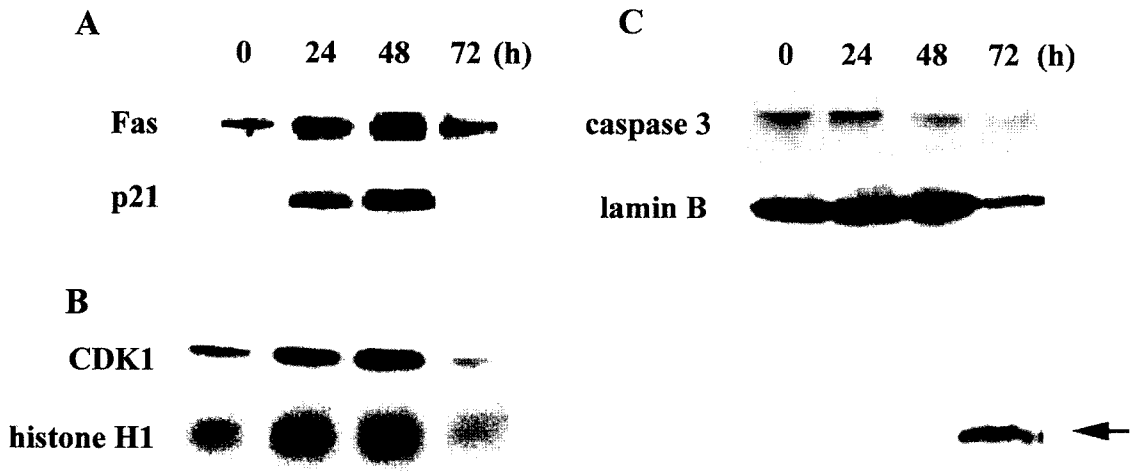

Fig. 2. Modulation in the expression of apoptosis-related proteins during VES-induced apoptosis in U937 cells. Whole cell lysates from the untreated or VES-treated U937 cells for indicated periods were analyzed by Western blotting. Immunoblots were probed with antibodies against Fas and p21Waf1 (A), CDK1 (B), and caspase-3 and lamin B proteins (C). For CDK1 activity assay, cell lysates were immunoprecipitated with anti-CDK1 antibody, and the *in vitro* kinase reactions were performed using histone H1. Arrow indicates the cleaved lamin B protein.

PKC-βII and the apoptosis induced by VES in U937 cells, caspase activity and DNA fragmentation were analyzed using a specific PKC inhibitor, GF109203X. When treated with VES, U937 cells exhibited an increase in caspase activity up to 9 fold at 72 h, which was greatly reduced by co-treatment with GF109203X (Fig. 4A). The VES-induced DNA fragmentation was completely blocked by GF109203X (Fig. 4B). These results demonstrated that PKC-βII activation was required for the VES-induced apoptosis.

We previously described that nitric oxide is an important intermediator during the VES-induced differentiation of U937 cells (Kim et al., 1998). To confirm the feasibility of involvement of NO in VES-induced apoptosis, the effect of GF109203X on NO production was analyzed. As shown in Fig. 5A, GF109203 had no effect on VES-induced NO production (Fig. 5A). Moreover, blockage of VES-induced NO production by co-

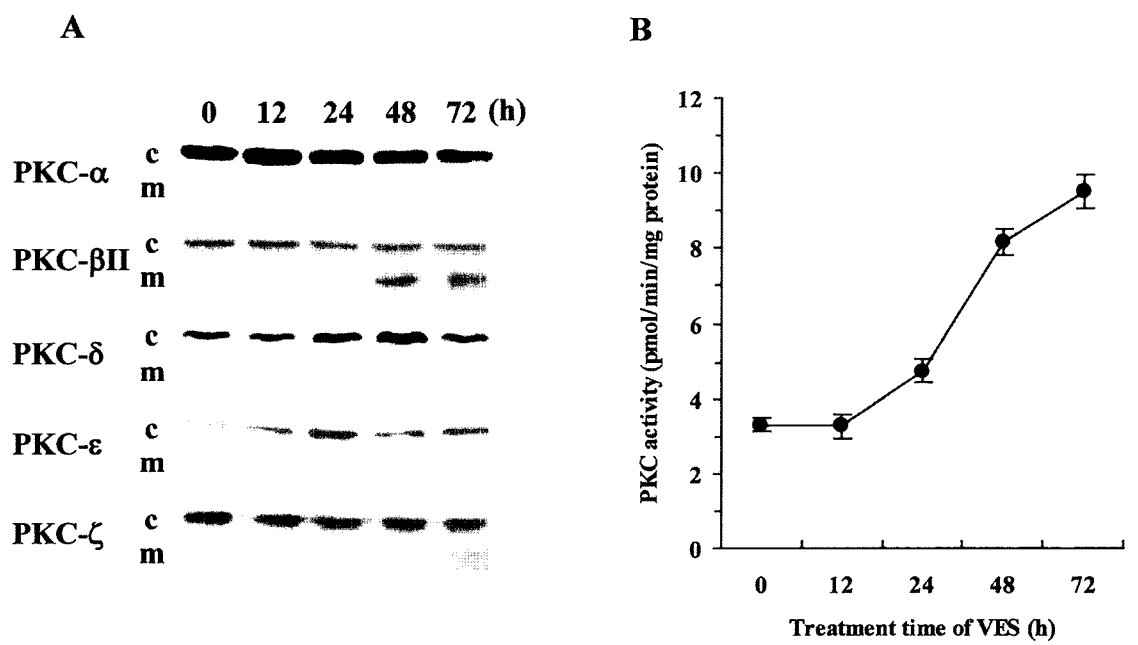

Fig. 3. VES-induced translocation of PKC-isoforms and activation of PKC. U937 cells were treated with 10 μg/ml VES for the indicated periods. A, Distribution of PKC-isozymes. Cells were sonicated and fractionated. Membrane and cytosolic fractions were anlayzed by Western blotting using respective antibodies of PKC isozymes. B, Activity of PKC was determined as described in 'Materials and Methods' using PS/DG-dependent transfer of [^{32}P]ATP to substrate.

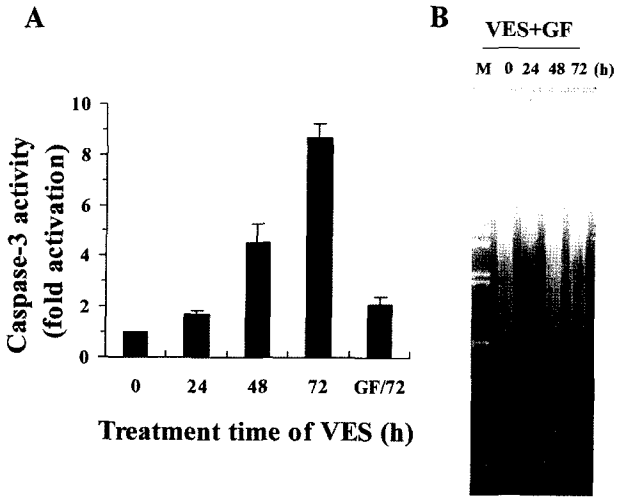

Fig. 4. Effect of GF109203X on the VES-induced apoptosis of U937 cells. Cells were cultured with 10 μg/ml VES or VES plus 1 μM GF109203X for the indicated periods. A, Caspase activity was measured by cleavage of the Ac-DEVD-AMC fluorogenic substrate. GF/72 indicates that the cells were co-treated with VES and GF109203X for 72 h. B, DNA fragmentation analysis was performd as in Fig. 1B.

treatment with L-NMMA, a NO synthase inhibitor, did not affect the VES-induced apoptosis (Fig. 5B). These results indicated that the VES-induced apoptosis through PKC activation occurs independently of NO production by VES.

Discussion

When cells are exposed to external insults, negative regulation of cell cycle or apoptosis occurs; cells which fail to repair DNA are eliminated through apoptosis (Lotem et al., 1991; Imamura et al., 1994). In this study, we have investigated the mechanisms of apoptosis induced by VES treatment in the human monocytic cell line U937. Our results indicate that VES induces apoptosis, at least in part, through activation of PKC-βII which stimulates caspase-3 cascade.

In this study, VES-treated U937 cells exhibited apoptotic characteristics, including nuclear DNA condensation and DNA fragmentation yielding an apoptotic body shape and ladder formation. VES also increased protein levels of Fas and p21. Fas (APO/CD95), a member of the tumor necrosis factor receptor, is known to trigger apoptosis in a number of different cell types upon ligand binding (Nagata, 1997; Turley et al., 1997; Yu et al., 1999). p21waf1/cip1 represents a family of molecules which block the activity of the cyclin/cdk complexes, and acts as one of the key regulatory proteins in cell cycle, terminal differentiation, and apoptosis (Frey et al., 1997). In contrast to the increase in p21 protein expression, VES treatment induced large increases in the expression and activation of CDK1 (also known as cdc2) in these cells. This aberrant expression and activation of CDK1, however, are consistent with what is associated with Fas induced apoptosis of hematopoietic cells (Furukawa et al., 1996). These data are also consistent with the result that unscheduled cyclin B/cdc2 activation takes place in response to DNA damage in HL-60 cells committed to apoptosis (Shimizu et al., 1995).

A common feature of cells undergoing apoptosis is activation of caspases, a family of the death-related cysteine proteases (Earnshaw et al., 1999). It was also reported that lamin cleavage participates in the activation of DNA fragmentation and nuclear apoptosis, and the process has been related to the roles of PKC isoforms (Lazebnik et al., 1993; Goss et al., 1994; Rao et al., 1996; Shimizu et al., 1998). Consistent with these reports, our study showed that VES induced the

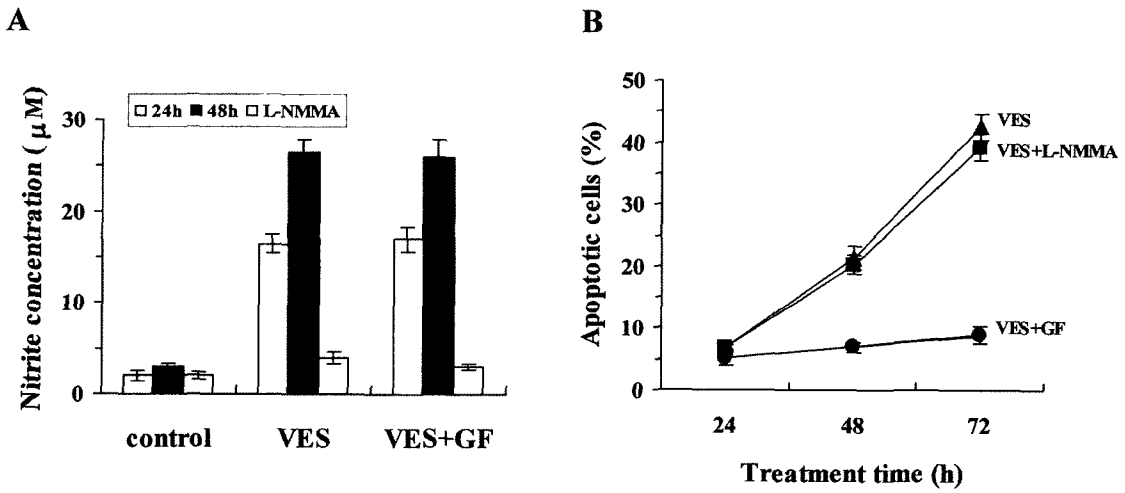

Fig. 5. Effects of NO and PKC inhibitor on the VES-induced apoptosis of U937 cells. Cells were cultured with 10 μg/ml VES, VES plus 1 μM GF109203X, or VES plus 2 mM L-NMMA for the indicated periods. A, Induction of NO by VES treatment was not affected by PKC inhibition. Release of NO was measured by the use of Griess reagent. L-NMMA (2 mM), a competitive NOS inhibitor was used for confirmation of the involvement of iNOS in NO production. B, The apoptotic fraction of the cell cycle was assessed by FACS analysis as described in 'Materials and Methods'. The data represent averages of four independent experiments with standard deviation.

cleavage and activation of caspase-3, followed by the cleavage of lamin B. VES also stimulated membrane translocation of PKC-βII and activation of PKC, and pretreatment of cells with the specific PKC inhibitor, GF109203X abrogated the DNA fragmentation induced by VES. Inhibition of PKC pathway also prevented the induction of caspase activity and apoptosis by VES. These results indicate that the VES- stimulated PKC signaling pathway is upstream of the caspase cascade, which triggers apoptosis by VES. Taken together, our data suggest that activation of PKC-βII plays a crucial role in apoptosis of these cells.

The effect of VES on the regulation of PKC activity is very complex. Although the mechanism of PKC regulation by VES is still obscure, several studies have shown that VES inhibits activation of PKC by changing its phosphorylation state as reported in smooth muscle cells and mesengial cells (Clement et al., 1997; Studer et al., 1997; Ricciarel et al., 1998). However, in most of those studies the results are due to the ability of VES as an antioxidant, and the inhibition of PKC activity by VES has been shown to be dependent on cell types.

Our study showed the opposing effect of VES treatment on the regulation of PKC activation; VES stimulated the membrane translocation of PKC-βII and activation of PKC, which induced caspase 3-mediated apoptosis. In our system the effect of VES on monocytic differentiation and apoptosis of U937 cells were not due to antioxidant function of VES as discussed in our previous report (Kim et al., 1998), and thus the precise signaling pathway leading to PKC activation by VES remained to be elucidated.

Acknowledgements

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References

Chang SH, Oh CD, Yang MS, Kang S-S, Lee YS, Sonn JK, and Chun JS (1998) Protein kinase C regulates chondrogenesis of mesenchymes via mitogen-activated protein kinase signaling. *J Biol Chem* 273: 19213-10219.

Clement S, Tasinato A, Boscoboinik D, and Azzi A (1997) The effect of alpha-tocopherol on the synthesis, phosphorylation and activity of protein kinase C in smooth muscle cells after phorbol 12-myristate 13-acetate down-regulation. *Eur J Biochem* 246: 745-749.

Cross T, Griffiths G, Deacon E, Sallis R, Gough M, Watters D, and Lord JM (2000) PKC-delta is an apoptotic lamin kinase. *Oncogene* 19: 2331-2337.

Earnshaw WC, Martins LM, and Kaufmann SH (1999) Mammalian caspases: structure, activation, substrates, and functions during apoptosis. *Annu Rev Biochem* 68: 383-424.

Frasch SC, Henson PM, Kailey JM, Richter DA, Janes MS, Fadok VA, and Bratton DL (2000) Regulation of phospholipid scrambles activity during apoptosis and cell activation by protein kinase c delta. *J Biol Chem* 275: 23065-23073.

Frey MR, Saxon ML, Zhao X, Rollins A, Evans SS, and Black JD (1997) Protein Kinase C isozyme-mediated cell cycle arrest involves induction of p21waf/cip1 and p27kip1 and hypophosphorylation of the retinoblastoma protein in intestinal epitherial cells. *J Biol Chem* 272: 9424-9435.

Furukawa Y, Iwase S, and Terui Y (1996) Transcriptional activation of the *cdc2* gene is associated with Fas-induced apoptosis of human hematopoietic cells. *J Biol Chem* 271: 28469-28477.

Goss VL, Hocevar BA, Thompson LJ, Stratton DJ, and Fields AP (1994) Identification of nuclear BII protein kinase C as a mitotic lamin kinase. *J Biol Chem* 269: 19074-19080.

Hug H and Sarre TF (1993) Protein kinase C isoenzymes: divergence in signal transduction? *Biochem J* 15: 329-343.

Imamura J, Miyoshi I, and Koeffler HP (1994) p53 in hematologic malignancies. *Blood* 84: 2412-2421.

Israel K, Yu W, Sanders BG, and Kline K (2000) Vitamin E succinate induces apoptosis in human prostate cancer cells: role for Fas in vitamin E succinate-triggered apoptosis. *Nutr Cancer* 36: 90-100.

Kim SJ, Bang OS, Lee Y-S, and Kang SS (1998) Production of inducible nitric oxide is required for monocytic differentiation of U937 cells induced by vitamin E-succinate. *J Cell Sci* 111: 435-441.

Lazebnik YA, Cole S, Cooke CA, Nelson WG, and Earnshaw WC (1993) Nuclear events of apoptosis in cell-free mitotic extracts: a model system for analysis of the active phase of apoptosis. *J Cell Biol* 123: 7-22.

Lotem J, Cragoe EJ Jr, and Sachs L (1991) Rescue from programmed cell death in leukemic and normal myeloid cells. *Blood* 15: 953-960.

Maciejewski JP Selleri C, Sato T, Cho HJ, Keeper LK, and Nathan CF (1995). Nitric oxide suppression of human hematopoiesis in vitro: contribution to inhibitory action of interferon-r and tumor necrosis factor-a. *J Clin Invest* 96: 1085-1092.

Mansat V, Laurent G, Levade T, Bettaieb A, and Jaffrezou JP (1997) The protein kinase C activators phorbol esters and phosphatidylserine inhibit neutral sphingomyelinase activation, ceramide generation, and apoptosis triggered by daunorubicin. *Cancer Res* 57: 5300-5304.

Nagata S (1997) Apoptosis by death factor. *Cell* 88: 355-365.

Neuzil J, Svensson I, Weber T, Weber C, and Brunk UT (1999) alpha-Tocopheryl succinate-induced apoptosis in Jurkat T cells involves caspase-3 activation, and both lysosomal and mitochondrial destabilisation. *FEBS Lett* 26: 445: 295-300.

Porn-Ares MI, Chow SC, Slotte JP, and Orrenius S (1997) Induction of apoptosis and potentiation of TNF- and Fas-mediated apoptosis in U937 cells by the xanthogenate compound D609. *Exp Cell Res* 25: 48-54.

Qian M, Kralova J, Yu W, Bose HR, Jr, Dvorak M, Sanders BG, and Kline K (1997) c-Jun involvement in vitamin E succinate induced apoptosis of reticuloendotheliosis virus transformed avian lymphoid cells. *Oncogene* 10: 223-230.

Rao L, Perez D, and White E (1996) Lamin proteolysis facilitates nuclear events during apoptosis. *J Cell Biol* 135: 1441-1455.

Riccirel R, Tasinato A, Clement S, Ozer NK, Boscoboinik D, and Azzi A (1998) alpha-Tocopherol specifically inactivates cellular protein kinase C alpha by changing its phosphorylattion state. *Biochem J* 15: 243-249.

Schutze S, Machleidt T, and Kronke M (1994) The role of diacylglycerol and ceramide in tumor necrosis factor and interleukin-1 signal transduction. *J Leukoc Biol* 56: 533-541.

Shao RG, Cao CX, and Pommier Y (1997) Activation of PKC alpha downstream from caspases during apoptosis induced by 7-hydroxystaurosporine or the topoisomerase inhibitors, camptothecin and etoposide, in human myeloid leukemia HL60 cells. *J Biol Chem.* 272: 31321-31325.

Shimizu T, Cao CX, Shao RG, and Pommier Y (1998) Lamin B phosphorylation by protein kinase calpha and proteolysis during apoptosis in human leukemia HL60 cells. *J Biol Chem* 273: 8669-8674.

Shimizu T, O'Connor PM, Kohn KW, and Pommier Y (1995)

Unschduled activation of cyclin B1/cdc2 kinase in human promyelocytic leukemia cell line HL-60 cells undergoing apoptosis induced by DNA damage. *Cancer Res* 55: 228-231.

Sokoloski JA, Hodnick WF, Mayne ST, Cinquina C, Kim CS, and Sartorelli AC (1997) Induction of the differentiation of HL-60 promyelocytic leukemia cells by vitamin E and other antioxidants in combination with low levels of vitamin D3: possible relationship to NF-kappaB. *Leukemia* 11: 1546-1553.

Studer RK, Craven PA, and DeRubertis FR (1997) Antioxidant inhibition of protein kinase C-signaled increases in transforming growth factor-beta in mesangial cells. *Metabolism* 46: 918-925.

Turley JM, Fu T, Ruscetti FW, Mikovits JA, Bertolette DC, and Birchenall-Roberts MC (1997) Vitamin E succinate induces Fas-mediated apoptosis in estrogen receptor-negative human breast cancer cells. *Cancer Res* 57: 881-890.

Yu W, Israel K, Liao QY, Aldaz CM, Sanders BG, and Kline K (1999) Vitamin E succinate (VES) induces Fas sensitivity in human breast cancer cells: role for Mr 43,000 Fas in VES-triggered apoptosis. *Cancer Res* 59: 953-961.

Yu W, Simmons-Menchaca M, You H, Brown P, Birrer MJ, Sanders BG, and Kline K (1998) RRR-alpha-tocopheryl succinate induction of prolonged activation of c-jun amino-terminal kinase and c-jun during induction of apoptosis in human MDA-MB-435 breast cancer cells. *Mol Carcinog* 22: 247-257.

Zhao B, Yu W, Qian M, Simmons-Menchaca M, Brown P, Birrer MJ, Sanders BG, and Kline K (1997) Involvement of activator protein-1 (AP-1) in induction of apoptosis by vitamin E succinate in human breast cancer cells. *Mol Carcinog* 19: 180-190.

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