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Biochemical Pharmacology 66 (2003) 273–280
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Immunosuppressant PG490 (triptolide) induces apoptosis through the activation of caspase-3 and down-regulation of XIAP in U937 cells

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Received 18 December 2002; accepted 7 April 2003

Abstract

PG490 (triptolide) is a natural, biologically active compound extracted from Chinese herb *Tripterygium wilfordii*. It possesses potent anti-inflammatory and immunosuppressive properties. The mechanism by which triptolide initiates apoptosis remains poorly understood. In the present report, we investigated the effect of triptolide on the apoptotic pathway in U937 human promonocytic cells. We show that triptolide inhibits U937 cells growth by inducing apoptosis. Following treatment of U937 cells with 25 nM triptoride for 24 hr, morphological features of apoptosis and DNA fragmentation were observed. Caspase inhibitors significantly reduced triptolide-induced caspase-3 activation. In addition, apoptosis triggered by triptolide was not associated with the generation of reactive oxygen species, which was not affected by the antioxidant *N*-acetylcysteine (NAC). The data collectively indicate that the cytotoxic effect of triptolide in U937 cells is attributable to apoptosis mediated by the caspase-3 activation pathway that may be associated with XIAP down-regulation.

Keywords: Triptolide; Apoptosis; U937; XIAP; Caspase

1. Introduction

Tripterygium wilfordii Hook. F (TWHF) has been used in traditional Chinese medicine for centuries. Extracts of TWHF have positive effects on autoimmune diseases, such as rheumatoid arthritis, nephritis and systemic lupus erythematosus [1–3]. A primary component of most functional TWHF extracts is triptolide [4]. Recent studies show that triptolide inhibits mitogen-induced lymphocyte proliferation and has immunosuppressive effects in skin allograft transplantation [5,6]. Further reports demonstrate that triptolide possesses antiproliferative activity against L1210 and P388 mouse leukemia cells and suppresses colony formation of breast cancer cell lines [4,7]. However, the cellular and molecular mechanisms underlying triptolide-induced apoptosis in leukemia cells are currently unclear.

Apoptosis is a process of genetically regulated cell death that plays an essential role in the development and maintenance of homeostasis, and protection against virus infection [8]. Failure to undergo apoptosis comprises one of the mechanisms of oncogenesis and chemoresistance of transformed cells [9]. The signaling pathway leading to apoptosis involves the sequential activation of cysteine proteases known as caspases, resulting in protein cleavage and breakdown of DNA molecules. The apoptotic cascade includes both initiator and effector caspases [10]. A central coordinating step in cell death induced by cytotoxic agents involves the release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol [11]. Cytoplasmic cytochrome *c* induces a caspase-3-activating complex comprising cytochrome *c*, Apaf-1, dATP, and procaspase-9 [12,13]. The pro-apototic Bcl-2 family proteins, Bax and Bak, are involved in cytochrome *c* release [14,15].

In the present study, we investigated whether apoptotic cell death is associated with the antitumor activity of triptolide in human leukemia U937 cells. Moreover, to elucidate the mechanisms underlying triptolide-induced apoptosis, we examined whether cell death is accompanied by the activation of the caspase cascade and prevented by antiapoptotic Bcl-2. Our results show that triptolide-induced apoptotic cell death is mediated by caspase-3 activation and

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0006-2952/03/$ – see front matter
doi:10.1016/S0006-2952(03)00282-X

cleavage of PLC-γ1, which is attenuated by overexpression of Bcl-2.

2. Materials and methods

2.1. Cells and materials

Human leukemia U937 cells were obtained from the American Type Culture Collection (ATCC). Dulbecco's modified Eagle's medium containing 10% fetal calf serum (FCS), 20 mM HEPES buffer, and 100 μg/mL gentamicin was employed throughout the experiments. Triptolide was directly added to cell cultures at the indicated concentrations. Anti-cIAP1, anti-cIAP2, anti-Bcl-2, anti-Bax, and anti-HSP70 antibodies were purchased from Santa Cruz Biotechnology Inc. Antibodies against specific proteins were obtained from the following suppliers: XIAP from R&D systems, and Asp-Glu-Val-Asp-chromophore *p*-nitroanilide (DEVD-pNA) and benzyloxycarbony-Val-Ala-Asp-fluoromethyl ketone (z-VAD-fmk) from Biomol. Triptolide was acquired from Alexis.

2.2. Western blotting

Cellular lysates were prepared by suspending 1.5×10^6 cells in 100 μL of lysis buffer (137 mM NaCl, 15 mM EGTA, 0.1 mM sodium orthovanadate, 15 mM $MgCl_2$, 0.1% Triton X-100, 25 mM MOPS, 100 μM phenylmethylsulfonyl fluoride, and 20 μM leupeptin, adjusted to pH 7.2). The cells were disrupted by sonication and extracted at 4 ° for 30 min. Proteins were electrotransferred to Immobilon-P membranes (Millipore Corporation). Specific proteins were detected with an ECL Western blotting kit, according to the manufacturer's instructions.

2.3. Cell count and flow cytometry analysis

Cell counts were performed using a hemocytometer. Approximately 1.5×10^6 U937 cells were suspended in 100 μL of PBS, and 200 μL of 95% ethanol were added while vortexing. Cells were incubated at 4° for 1 hr, washed with PBS, and resuspended in 250 μL of 1.12% sodium citrate buffer (pH 8.4), together with 12.5 μg RNase. Incubation was continued at 37° for 30 min. Cellular DNA was stained by applying 250 μL propidium iodide (50 μg/mL) for 30 min at room temperature. Using a FACScan flow cytometer, stained cells were analyzed for the relative DNA content based on red fluorescence.

2.4. DNA fragmentation assay

After treatment with triptolide, U937 cells were lysed in buffer containing 10 mM Tris (pH 7.4), 150 mM NaCl, 5 mM EDTA, and 0.5% Triton X-100 for 30 min on ice. Lysates were vortexed and cleared by centrifugation at 10,000 *g* for 20 min. Fragmented DNA in the supernatant was extracted with an equal volume of neutral phenol:-chloroform:isoamyl alcohol mixture (25:24:1), and analyzed electrophoretically on 2% agarose gels containing 0.1 μg/mL ethidium bromide.

2.5. Caspase-3 activity assay

To evaluate caspase-3 activity, cell lysates were prepared following treatment with triptolide. Assays were performed in 96-well microtitre plates by incubating 20 μg cell lysates in 100 μL reaction buffer (1% NP-40, 20 mM Tris–HCl, pH 7.5, 137 mM NaCl, 10% glycerol) containing 5 μM DEVD-pNA (a caspase-3 substrate). Lysates were incubated at 37° for 2 hr. Absorbance at 405 nm was measured with a spectrophotometer.

2.6. RNA isolation and reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was isolated according to the procedure of Chomczynski and Sacchi [16]. Single-strand cDNA was synthesized from 2 μg total RNA using M-MLV reverse transcriptase (Gibco-BRL). XIAP and actin cDNA were amplified by PCR with specific primers. The following sense and antisense primers were employed for XIAP: 5′-CTTGAGGAGTGTCTGGTAAG-3′ and 5′-GTGACTA-GATGTCCACAAGG-3′, respectively. Sense and antisense primers for cIAP1 were 5′-CCTGACAACTGGAGA-GAACT-3′ and 5′-ACTACCAGATGACCACAAGG-3′, while those for cIAP2 were 5′-ACCTACCTGTGGA-GATGCCT-3′ and 5′-CCAGCACGAGCAAGACTCCT-3′, respectively. PCR conditions employed were: 1× (94°, 3 min), 30× (94°, 45 s; 58°, 45 s; and 72°, 1 min), and 1× (72°, 10 min). PCR products were analyzed by agarose gel electrophoresis and visualized using ethidium bromide.

2.7. DNA transfection and luciferase assay

Chromosomal DNA was prepared from U937 cells using the DNAzol™ reagent (Gibco-BRL). Human XIAP promoter was amplified from chromosomal DNA with the following synthetic primers: 5′-AAGGCAAAA-GAGCTCGCTAATTC (sense), and 5′-GCAGGTACA-CAAGCTTTAGAT (antisense). The PCR product was digested with *Sac*I and *Hin*dIII and cloned upstream of the firefly luciferase gene of pGL2-basic (Promega). PCR products were confirmed by their size, as determined by electrophoresis and DNA sequencing. The XIAP promoter plasmid was transfected into human embryonic kidney (HEK) 293 cells using the Lipofectamine reagent (Life Technologies) according to the manufacturer's instructions. To assess XIAP promoter luciferase activity, cells were collected and disrupted by sonication in lysis buffer (25 mM Tris–phosphate, pH 7.8, 2 mM EDTA,

1% Triton X-100, and 10% glycerol). After centrifugation, aliquots of supernatants were tested for luciferase activity using the luciferase assay system (Promega), as specified by the manufacturer.

3. Results

3.1. Cytotoxic effect of triptolide on U937 cell death

To verify triptolide-induced cell toxicity, we examined the effects of different concentrations of triptolide on U937 cell viability. After 24 hr of treatment, survival was inversely correlated with triptolide concentration. Significant loss of viability was detected at 25 or 75 nM triptolide. Specifically, following treatment with triptolide (25 or 75 nM) for 24 hr, 43 and 20% cells survived in culture, respectively (Fig. 1A). These results indicate that cell viability in the presence of triptolide decrease in a dose- and time-dependent manner.

To evaluate the nature of the induced apoptosis, cells treated for 24 hr with 75 nM triptolide were examined after propidium iodine staining. Nucleic acid staining with propidium iodine revealed typical apoptotic nuclei in cells treated with triptolide in contrast to control cells, which did not display any features of apoptosis (Fig. 1B). Another hallmark of apoptosis is degradation of chromosomal DNA at internucleosomal linkages. Accordingly, we analyzed whether DNA fragmentation was induced by triptolide in U937 cells. Following agarose gel electrophoresis of U937 cells treated with various concentrations of triptolide for 24 hr, a typical ladder pattern of internucleosomal fragmentation was observed (Fig. 1C).

To quantify the degree of apoptosis, we analyzed the amount of sub-G_1 DNA by flow cytometry of fixed nuclei (Fig. 2A). U937 cells were exposed to various concentrations of triptolide for 24 hr. As shown in Fig. 2B, the addition of triptolide to U937 cells resulted in markedly increased accumulation of the sub-G_1 phase in a dose-dependent manner.

3.2. Caspase mediates triptolide-induced apoptosis

Recent studies have identified caspases as important mediators of apoptosis induced by various apoptotic stimuli [10]. To determine the roles of caspases in triptolide-induced apoptosis, we measured the activity of caspase-3 in U937 cells treated with triptolide. Caspase-3 is activated by proteolytic processing of the 32-kDa form into two smaller subunits. Caspase-3 activity during triptolide-induced apoptosis was determined by measuring proenzyme levels using Western blot analysis, and proteolytic

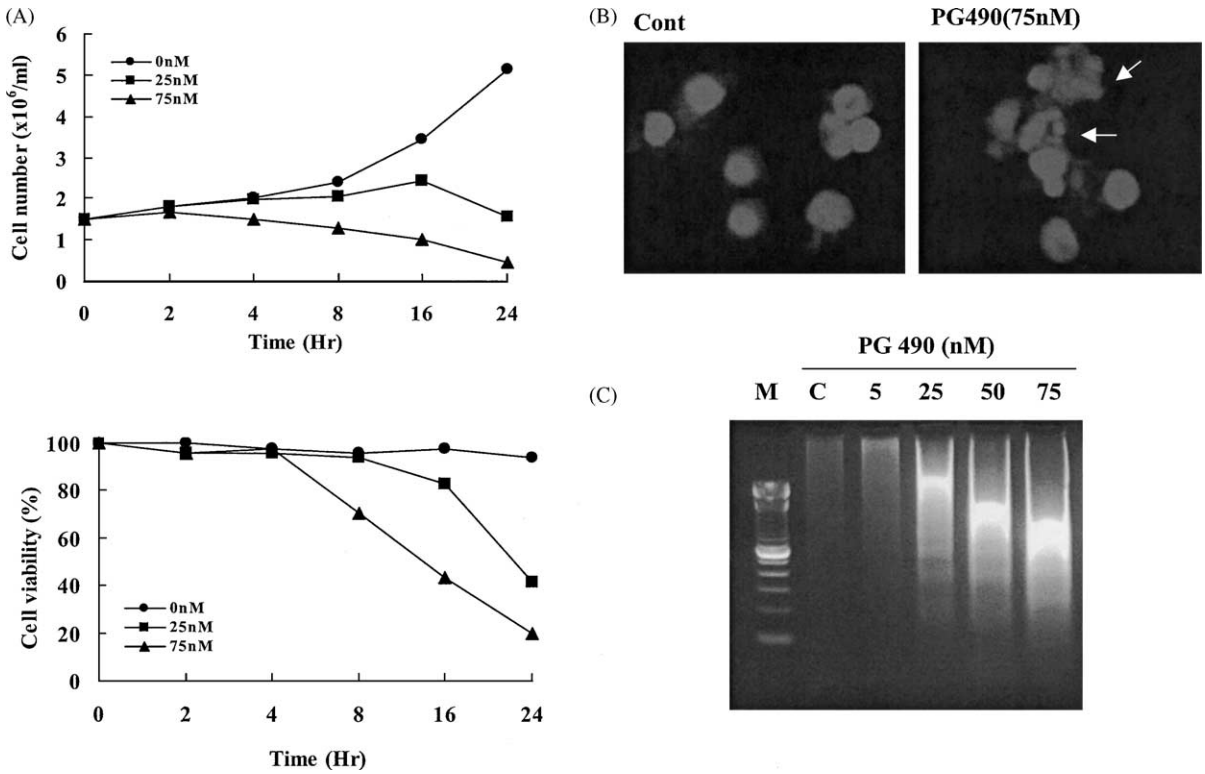

Fig. 1. Effect of triptolide on U937 cell growth rate, viability, morphological characteristics, and DNA fragmentation. (A) U937 cells were treated with indicated concentrations of triptolide for various time-periods. Cell death was determined using the trypan blue exclusion assay. (B) Morphological aspects of propidium iodide-stained cells. U937 cells were treated with 75 nM triptolide for 24 hr, and stained with propidium iodide. The arrows indicate condensed or fragmented nuclei. (C) To analyze fragmentation of genomic DNA, cells were treated for 24 hr with the indicated concentrations of triptolide. Fragmented DNA was extracted and analyzed on a 2% agarose gel.

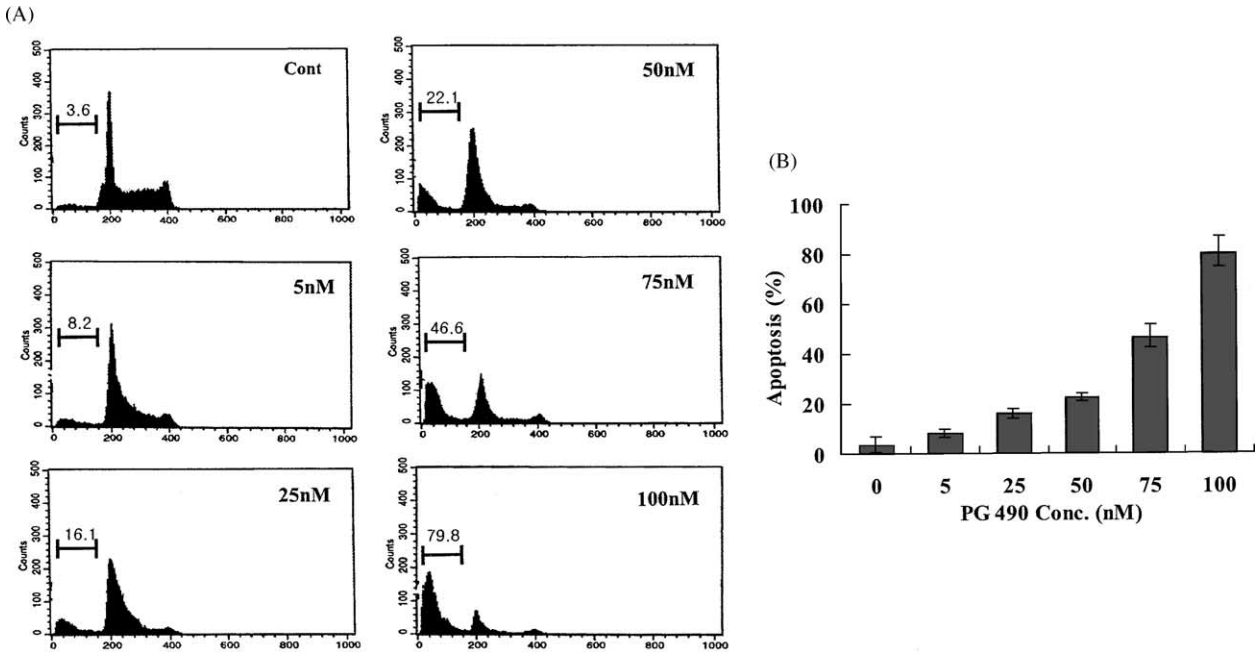

Fig. 2. Flow cytometric analysis of apoptotic cells. (A) Cells were treated for 24 hr with the indicated concentrations of triptolide, and evaluated for DNA content after propidium iodide staining. (B) The fraction of apoptotic cells is indicated. Data are presented as the mean values obtained from three independent experiments, and bars represent standard deviation.

activity with a chromogenic substrate. As shown in Fig. 3A, treatment of U937 cells with 25–100 nM triptolide for 24 hr resulted in a decrease in pro-caspase-3 levels. Caspase-3 is one of the key proteases responsible for the cleavage of poly (ADP-ribose) polymerase (PARP), DNA-dependent protein kinase, protein kinase C δ (PKC-δ) and Bax, among other substrates [17–20]. PLC-γ1 is cleaved into a 60-kDa fragment by *in vivo* activated caspase-3 [21]. Subsequent Western blotting disclosed proteolytic cleavage of PLC-γ1 in U937 cells after 24 hr of treatment with 25 nM triptolide. PLC-γ1 cleavage was dose-dependent in U937 cells (Fig. 3A). To further quantify the proteolytic activity of caspase-3, we performed an *in vitro* assay, based on the cleavage of DEVD-pNA by caspase-3 into chromophore *p*-nitroanilide (pNA). U937 cells displayed a 4.2-fold increase in DEVD-pNA cleavage after 24 hr exposure to 75 nM triptolide (Fig. 3B).

To assess the significance of caspase activation in triptolide-induced apoptosis, we used a general and potent inhibitor of caspases, z-VAD-fmk. Triptolide strongly stimulated caspase-3 protease activity, which was abolished in cells pretreated with z-VAD-fmk (Fig. 3C). Furthermore, triptolide treatment of U937 cells resulted in the generation of a 60-kDa cleavage product of PLC-γ1. Notably, z-VAD-fmk-pretreated cells displayed significantly decreased PLC-γ1 cleavage (Fig. 3C). Blockage of caspase activity by pretreatment of U937 cells with 50 μM z-VAD-fmk prevented triptolide-induced genomic DNA digestion (Fig. 3D). Our data clearly demonstrate that triptolide-induced apoptosis is associated with caspase activation.

3.3. Induction of apoptosis by triptolide appears to be independent of formation of reactive metabolites

Many antineoplastic agents eliminate tumor cells by inducing programmed cell death or apoptosis, and numerous investigations have documented cellular changes resulting from oxidative stress induced in cells following exposure to cytotoxic drugs and UV and γ irradiation [22,23]. To determine whether reactive oxygen species (ROS) play a role in triptolide-induced apoptosis, we used the thiol antioxidant, NAC, which functions both as a redox buffer and reactive oxygen intermediate scavenger [24]. NAC was added to 75 nM triptolide-treated U937 cells at concentrations of 5–10 mM. No significant changes in caspase-3 activity, levels of pro-caspase-3, or cleavage of PLC-γ1 were noted after exposure to NAC plus 75 nM triptolide for 24 hr (Fig. 4). These data clearly indicate that triptolide-induced apoptosis is not associated with ROS generation.

3.4. Overexpression of Bcl-2 prevents triptolide-mediated caspase-3 activation

To analyze whether triptolide induces cell death by modulating the expression of Bcl-2 family members, we determined Bcl-2 levels in U937 cells exposed to various concentrations of triptolide. U937 cells treated with triptolide at concentrations sufficient to induce apoptosis did not display altered expression of Bcl-2 or Bax proteins after 24 hr (Fig. 5A). Our data imply that Bcl-2 and Bax

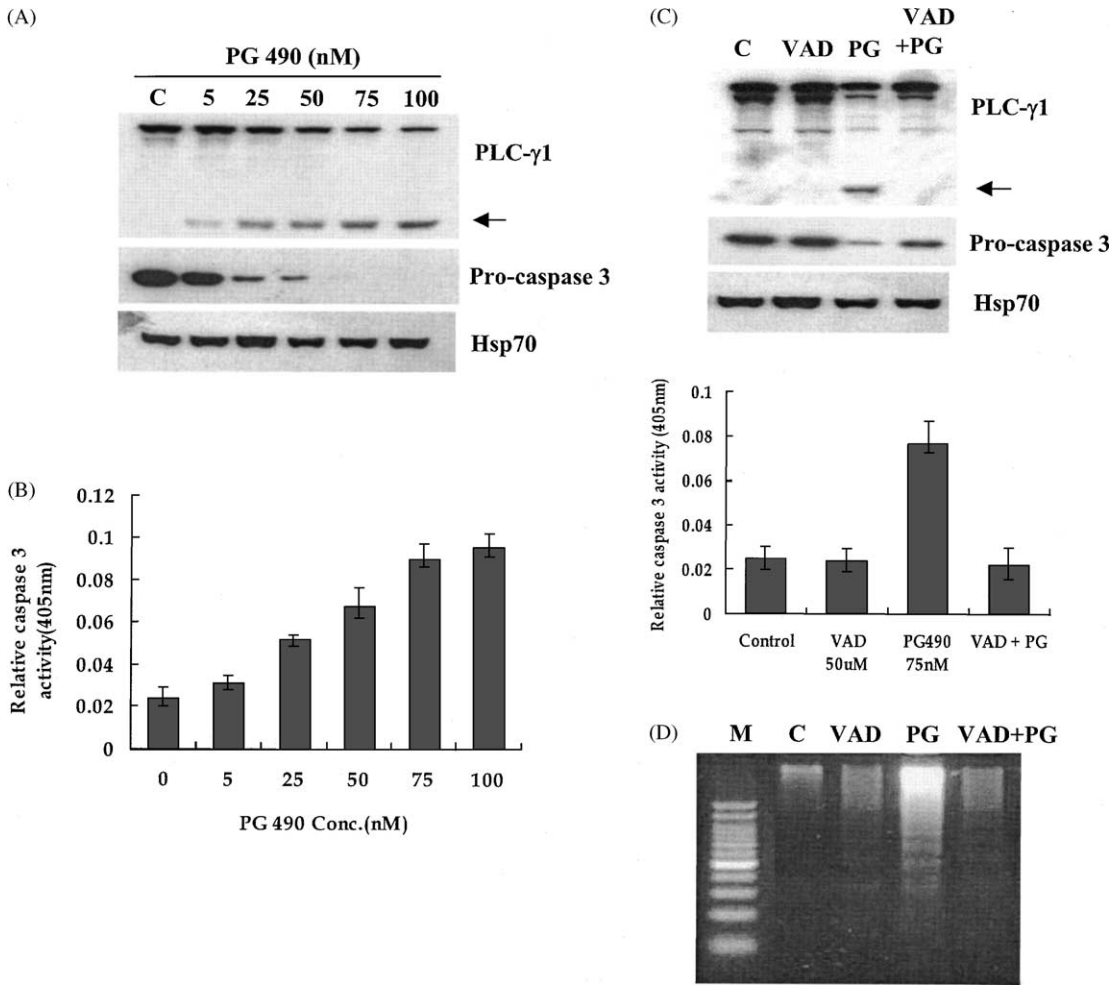

Fig. 3. Effect of triptolide on caspase-specific cleavage of PLC-γ1 and caspase-3 activity. (A) Cells were treated with the indicated concentrations of triptolide. Equal amounts of cell lysates (40 μg) were subjected to electrophoresis and analyzed by Western blotting to assess level of caspase-3 and PLC-γ1. Proteolytic cleavage of PLC-γ1 is specified with arrows. To confirm equal loading, the blot was stripped of bound antibody and reprobed with anti-Hsp70 antibody. (B) U937 cells were treated with indicated concentrations of triptolide for 24 hr and harvested in lysis buffer. Enzymatic caspase-3 activity was determined by incubating 20 μg total protein with 200 μM chromogenic substrate (DEVD-pNA) in a 100 μL assay buffer for 2 hr at 37°. The release of chromophore *p*-nitroanilide (pNA) was monitored spectrophotometrically (405 nm). Data are presented as means ± SD (N = 3). (C) Effects of z-VAD-fmk on triptolide-induced caspase-3 activation. U937 cells were incubated with z-VAD-fmk or solvent for 1 hr before treatment with triptolide. Equal amounts of cell lysates (40 μg) were subjected to electrophoresis and analyzed by Western blotting to determine level of caspase-3 and PLC-γ1. Caspase activity was determined as described above. To confirm equal loading, the blot was stripped of bound antibody and reprobed with anti-Hsp70 antibody. (D) Inhibition of triptolide-induced genomic DNA fragmentation by z-VAD-fmk. Genomic DNA was extracted and analyzed by 2% agarose gel electrophoresis.

protein levels have no effect on apoptosis induced by triptolide in U937 cells.

Although triptolide does not affect Bcl-2 expression, Bcl-2 is a well-known antiapoptotic protein. To evaluate the effect of high intracellular levels of Bcl-2 on caspase-3 activation and PLC-γ1 degradation during triptolide-induced apoptosis, we employed U937/vector and U937/Bcl-2 cells generated by transfection of Bcl-2 cDNA. As shown in Fig. 5B, U937/Bcl-2 cells exhibited a 4-fold increase in Bcl-2 expression, compared to cells containing the empty vector. Exposure to high doses of triptolide for 24 hr induced apoptosis in U937 cells. We measured pro-caspase-3 levels and activity of caspase-3 in U937/vector and U937/Bcl-2 cells exposed to 75 nM and 100 nM triptolide for 24 hr (Fig. 5C). Triptolide induced a decrease in the levels of pro-caspase-3 and high caspase-3 activity in U937/vector cells. In contrast, levels of pro-caspase-3 were slightly decreased in U937/Bcl-2 cells treated with triptolide. After 24 hr exposure to 75 nM triptolide, U937/vector cells exhibited a significant increase in caspase-3 activity, while only a slight increase in activity was observed in U937/Bcl-2 cells. Western blotting data revealed proteolytic cleavage of PLC-γ1 in U937/vector cells after 24 hr of triptolide treatment. In contrast, U937/Bcl-2 cells contained significantly reduced levels of the 60-kDa cleavage product (Fig. 5D). These data indicate that triptolide does not affect regulation of Bcl-2, overexpression of this protein attenuates triptolide-stimulated apoptosis through by caspase-3 inactivation.

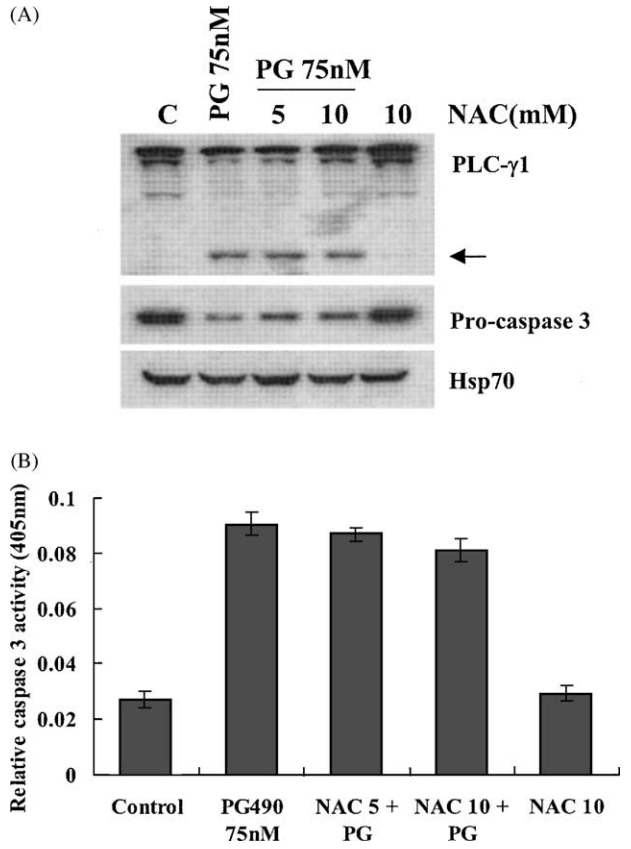

Fig. 4. Effect of *N*-acetylcysteine (NAC) on triptolide-induced caspase-3 activity and cleavage of PLC-γ1. (A) U937 cells were incubated with the indicated concentrations of NAC for 1 hr before treatment with triptolide (75 nM) for 20 hr. Equal amounts of cell lysates (40 μg) were resolved by SDS–PAGE, transferred to a nitrocellulose membrane, and probed with anticaspase-3 and anti-PLC-γ1 antibodies. To confirm equal loading, the blot was stripped of the bound antibody and reprobed with anti-Hsp70 antibody. (B) Caspase activity was determined as described in Fig. 3. Data are presented as means ± SD (N = 3).

3.5. Modulation of IAP protein families in triptolide-induced apoptosis

To determine whether caspase-3 activity in triptolide-induced apoptosis is associated with the levels of caspase inhibitors, we examined the expression of IAP family proteins in U937 cells exposed to various concentrations of triptolide. As shown in Fig. 6A, treatment of U937 cells with 25–100 nM triptolide for 24 hr resulted in decreased levels of cIAP1 and XIAP, but not cIAP2. The data indicate that elevated caspase-3 activity in triptolide-treated U937 cells is correlated to down-regulation of cIAP1 and XIAP, but not cIAP2.

To further elucidate the mechanism regulating changes in IAP family protein quantities, we analyzed XIAP, cIAP1, and cIAP2 mRNA levels by RT-PCR. Treatment with triptolide resulted in a marked decrease in XIAP and cIAP1 mRNA, but not cIAP2 or actin mRNA (Fig. 6B). To determine whether triptolide-mediated XIAP suppression is due to promoter activity, transient transfection of the XIAP reporter gene construct was performed. Triptolide significantly decreased promoter activity in a dose-dependent manner (Fig. 6C). A significant correlation between XIAP protein and mRNA levels was observed. Our results imply that triptolide-mediated XIAP expression is regulated, in part, at the transcriptional level.

4. Discussion

In the present study, we have demonstrated that triptolide induces apoptosis in association with the activation of caspase-3 and down-regulation of cIAP1 and XIAP, but not Bcl-2 family proteins. Furthermore, we have shown that Bcl-2 overexpression attenuates triptolide-induced apoptosis via inhibition of caspase-3 activity. Another important finding is that this apoptotic process is not mediated by ROS generation. Additionally, we found that inhibition of caspase activation by z-VAD-fmk prevents triptolide-induced apoptosis.

We have shown that triptolide-induced apoptosis regulated through caspase activation. Caspase-3 is an important cell death-inducing protease that cleaves PARP, PLC-γ1, and other vital proteins [17,18,21]. In our experiments, triptolide induced caspase-3 activation and PLC-γ1 cleavage in association with apoptosis. Furthermore, the caspase inhibitor, z-VAD-fmk, effectively blocked cleavage of PLC-γ1, activation of caspase-3, and DNA fragmentation. A possible cause for caspase-3 activation in triptolide-treated U937 cells is the decreased expression of IAP protein families. Human IAP proteins, including XIAP, c-IAP1, c-IAP2, and survivin, are characterized by the presence of one to three copies of the BIR domain, a 70-amino acid motif that bears homology to sequences found in the baculovirus IAP proteins [25]. These proteins reportedly block apoptosis due to their function as direct inhibitors of activated effector caspases (caspase-3 and caspase-7). Furthermore, cIAP1 and cIAP2 inhibit cytochrome *c*-induced activation of caspase-9 [26,27].

Members of the Bcl-2 family of proteins are associated with the mitochondrial membrane and regulate its integrity [28]. In our experiments, Bcl-2 protein levels did not affect response to triptolide. However, overexpression of this protein attenuated triptolide-induced apoptosis via inhibition of caspase-3 activity. The mechanisms underlying the triptolide-mediated function of Bcl-2 are unclear at present. Here we show that analogous to Bcl-2, Bax protein levels are not affected by triptolide. Bax is predominantly present in the cytosol. This protein exerts proapoptotic activity, is translocated from cytosol to mitochondria and induces cytochrome *c* release. Bcl-2 exerts antiapoptotic activity, partly by inhibiting the translocation of Bax to mitochondria [29].

Oxidative stress is suggested as a common mediator of apoptosis [30]. However, we report here that triptolide does not induce oxidative DNA damage and apoptosis in U937

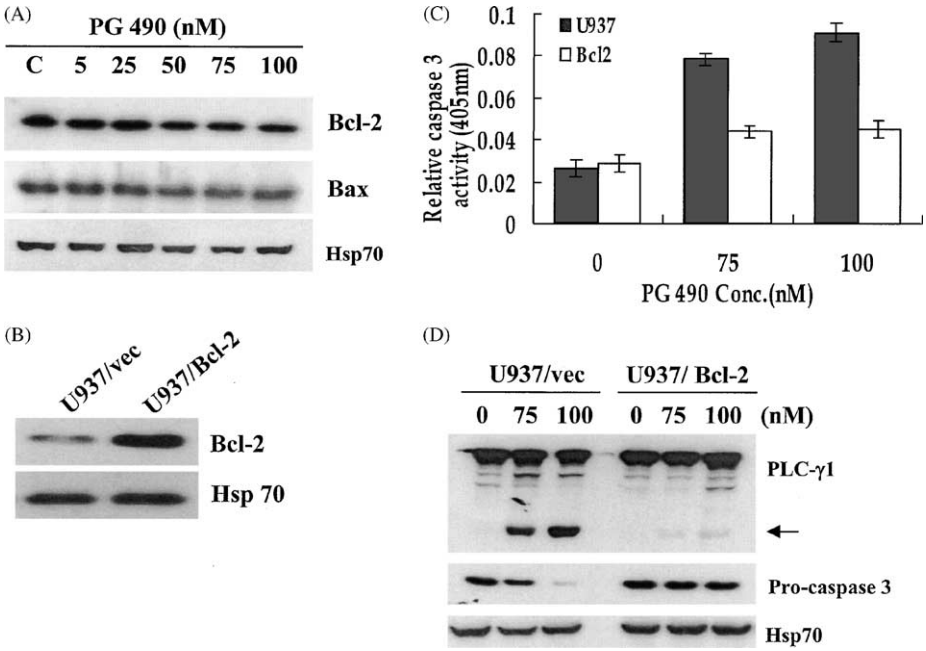

Fig. 5. Effect of Bcl-2 overexpression on triptolide-induced apoptosis. (A) U937 cells were treated with the indicated concentrations of triptolide. Equal amounts of cell lysates (40 μg) were resolved by SDS–PAGE, transferred to nitrocellulose membrane, and probed with specific antibodies (anti-Bcl-2 and anti-Bax). (B) Overexpression of Bcl-2 in U937 cells. Immunoblot analysis of cell lysates (40 μg) from control (U937/vector) and Bcl-2 transfected (U937/Bcl-2) cells. The blot was probed with anti-Bcl-2. To confirm equal loading, the blot was stripped of the bound antibody and reprobed with anti-Hsp70 antibody. (C) Effect of triptolide on caspase-3 activity in U937/vector and U937/Bcl-2 cells. Cells were treated with the indicated concentrations of triptolide. Caspase-3 activity was determined as described for Fig. 3. Data are presented as mean values from three independent experiments, and bars represent standard deviations. (D) Effect of triptolide on caspase-specific cleavage of PLC-γ1 in U937/vector and U937/Bcl-2 cells. Equal amounts of cell lysates (40 μg) were subjected to electrophoresis and analyzed by Western blotting to assess of caspase-3 and PLC-γ1. To confirm equal loading, the blot was stripped of the bound antibody and reprobed with anti-Hsp70 antibody.

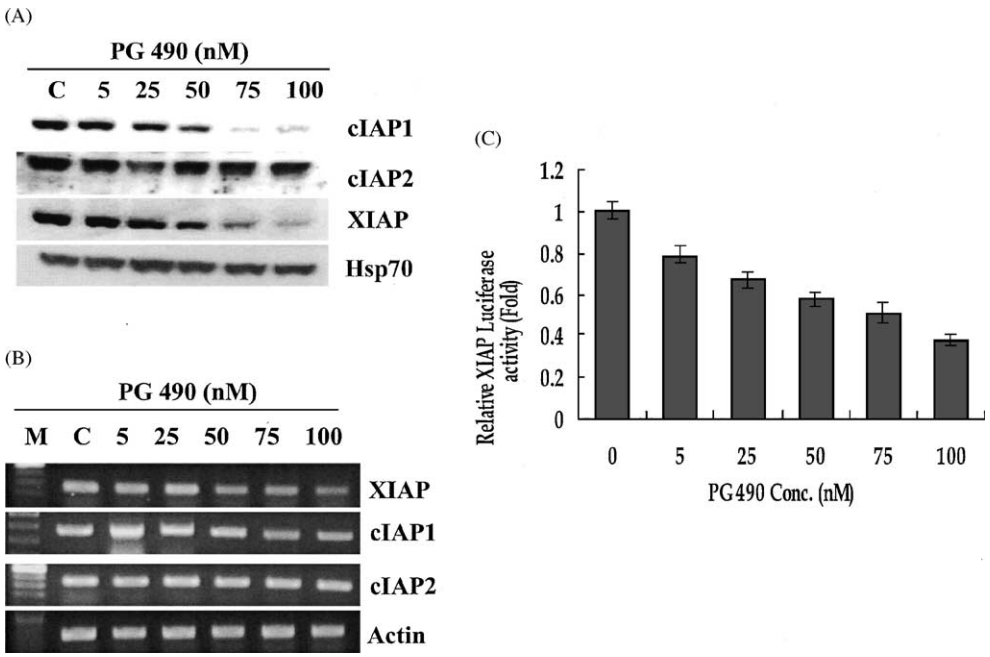

Fig. 6. Expression of apoptosis-related proteins in U937 cells following treatment with triptolide. (A) U937 cells were treated with the indicated concentrations of triptolide. Equal amounts of cell lysates (40 μg) were resolved by SDS–PAGE, transferred to nitrocellulose, and probed with specific antibodies (anti-cIAP1, anti-cIAP2, or anti-XIAP). To confirm equal loading, the blot was stripped of the bound antibody and reprobed with anti-Hsp70 antibody. A typical result is shown. Two additional experiments yielded similar results. (B) XIAP and cIAP1 mRNA down-regulation as a result of triptolide treatment. Cells were incubated for 16 hr with various concentrations of triptolide, and RT-PCR analysis was performed. XIAP and cIAP1 mRNA, but not cIAP2, decreased in a concentration-dependent manner. (C) Suppression of XIAP promoter activity by triptolide. A human XIAP promoter construct was transfected into HEK 293 cells, followed by incubation with the indicated concentrations of triptolide for 24 hr. Cells were harvested and assayed for luciferase activity. Data are presented as the mean values obtained from three independent experiments, and the bars represent standard deviations.

cells, based on the finding that the antioxidant, NAC, does not prevent apoptosis by triptolide.

In summary, triptolide is a novel drug with potent antiproliferative and anti-inflammatory effects in cancer cells. Our results suggest that triptolide induces apoptosis in cancer cells. Moreover, caspase-3 activation and down-regulation of the caspase inhibitory protein, XIAP, are involved in this apoptotic process. In view of the accumulating evidence that triptolide is an important determinant of clinical response to cancer, further efforts to explore this therapeutic strategy are warranted.

Acknowledgments

This study was supported by a grant (02-PJ9-PG1-CO04-0009) of the Oriental Medicine R&D Project, Ministry of Health & Welfare, Republic of Korea.

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