Inhibition of cyclooxygenase-2 and telomerase activities in human leukemia cells by dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp.

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Abstract. Dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp., is known to exhibit significant selective cytotoxic activity against several human cancer cell lines. In the present study, we investigated further possible mechanisms by which dideoxypetrosynol A exerts its anti-proliferative action in cultured human leukemia U937 cells. Exposure of U937 cells to dideoxypetrosynol A resulted in growth inhibition and induction of apoptosis as measured by hemocytometer counts, fluorescent microscopy, agarose gel electrophoresis and flow cytometry analysis. The increase in apoptosis was associated with a dose-dependent up-regulation in pro-apoptotic Bax expression and activation of caspase-3 and caspase-9. Dideoxypetrosynol A decreased the levels of cyclooxygenase (COX)-2 mRNA and protein expression without significant changes in the levels of COX-1, which was correlated with a decrease in prostaglandin E_2 (PGE_2) synthesis. Furthermore, dideoxypetrosynol A treatment markedly inhibited the activity of telomerase, and the expression of human telomerase reverse transcriptase (hTERT), a main determinant of the telomerase enzymatic activity, was progressively down-regulated by dideoxypetrosynol A treatment in a dose-dependent fashion. Taken together, these findings provide important new insights into the possible molecular mechanisms of the anti-cancer activity of dideoxypetrosynol A.

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Introduction

The arachidonic acid pathway is of particular importance to the etiology of cancer. Arachidonic acid, a 20-carbon polyunsaturated fatty acid, is a phospholipid component of cell membranes. Two key enzymes in arachidonic acid metabolism are cyclooxygenase-1 (COX-1) and -2 (COX-2); the former is the constitutive isoform while the latter is inducible. An overproduction of COX-2 enzyme that catalyzes arachidonic acid metabolism to form prostaglandin E_2 (PGE_2) facilitates proliferation of neoplastic cells (1-3). Moreover, compelling evidence from genetic and clinical studies indicates that COX-2 up-regulation is a key step in carcinogenesis and there is a clear positive correlation between COX-2 expression and the inhibition of cell proliferation (3,4). Overexpression of COX-2 is sufficient to cause tumorigenesis in animal models and the inhibition of the COX-2 pathway results in the reduction of tumor incidence and progression (5,6).

It is also increasingly clear that the process of neoplasia is characterized by the activation of telomerase that adds telomeric repeats to the ends of replicating chromosomes, telomeres (7). Telomeres are essential units that prevent the loss of genetic information. In normal somatic cells, which show little or no telomerase activity to synthesize new telomeres, the telomeric DNAs progressively shorten with each cell division. Critically short telomeres are suggested to cause irreversible cell growth arrest and cellular senescence (8). Conversely, most tumor cells have mechanisms that compensate for telomere shortening, most commonly through the activation of telomerase, allowing them to stably maintain their telomeres and grow indefinitely. These observations suggest that telomerase reactivation is a rate-limiting step in cellular immortality and carcinogenesis, and telomerase repression can act as a tumor-suppressive mechanism. Telomere length in human is primarily controlled by three major components; the human telomerase RNA (hTR), telomerase-associated protein-1 (TEP-1) and the human telomerase reverse transcriptase (hTERT). There is a good correlation between expression of hTERT mRNA and the presence of telomerase activity in extracts from tissue culture cells and normal and cancer tissues (9,10). These observations suggest that control of

the hTERT expression at the mRNA level mainly contributes to the regulation of telomerase enzymatic activity.

Recent studies indicated that marine organisms are proving to be a novel and rich source of bioactive compounds. Among them, marine sponges have been used as a main source for this study of hitherto unknown biological activities of natural marine products (11,12). Investigations into components of marine products have proven that many are not general cytotoxic agents but rather are targeted towards specific cellular or biochemical events and therefore hold a strong potential as antimicrobial, anticancer or anti-inflammatory agents (13-15). Through screening of natural compounds that inhibit cancer cell proliferation, we previously reported that dideoxypetrosynol A, a polyacetylene from the sponge *Petrosia* sp., has significant selective cytotoxic activity against a small panel of human solid tumor cell lines by inhibiting DNA replication and apoptotic cell death (15-18), however, the molecular mechanisms of its anti-proliferative action on malignant cell growth are not clear. To further explore the mechanism of its anticancer activity, we investigated its effects on human leukemia U937 cells. Our study revealed that dideoxypetrosynol A exhibited a significant inhibition on the growth of U937 cells in parallel with the reduction of COX-2 and hTERT which was associated with an inhibition of PGE_2 release and telomerase activity. These results suggested that dideoxypetrosynol A is a potential agent for cancer treatment.

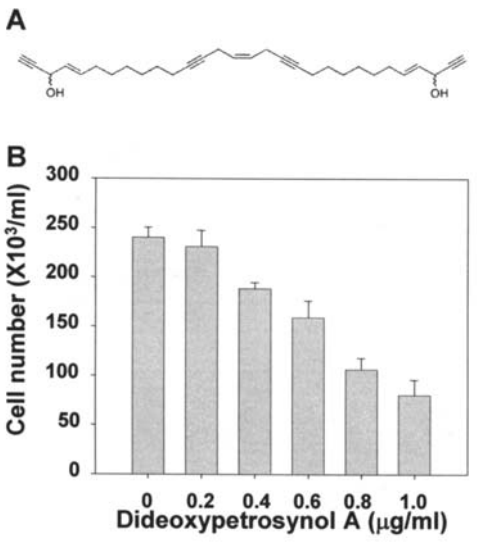

Figure 1. Effect of dideoxypetrosynol A on the viability of human leukemia U937 cells. (A) Chemical structure of dideoxypetrosynol A. (B) Cells were seeded at $3x10^4$ per ml in 35-mm dish and incubated for 24 h. The cells cultured in the absence (0, control) or in the presence of increasing concentrations of dideoxypetrosynol A for 48 h. Viable cell number was determined by hemocytometer counts of trypan blue-excluding cells. Data are the mean of two different experiments.

Materials and methods

Cell culture, dideoxypetrosynol A and cell viability study. The human leukemia cell line U937 was purchased from the American Type Culture Collection (Rockville, MD), and maintained at 37˚C in a humidified condition of 95% air and 5% CO_2 in DMEM (Gibco BRL, Gaithersburg, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Dideoxypetrosynol A (Fig. 1A) was prepared as described previously (16) and dissolved in dimethyl sulfoxide as a stock solution at 10 mg/ml concentration, and stored in aliquots at -20˚C. For the viability study, cells were cultured in the absence and presence of variable concentrations of dideoxypetrosynol A for 48 h. The cells were trypsinized, washed with phosphate-buffered saline (PBS) and the viable cells were scored with hematocytometer through the exclusion of trypan blue.

Nuclear staining with DAPI. Cells were washed with PBS and fixed with 3.7% paraformaldehyde (Sigma Chemical Co., St. Louis, MO) in PBS for 10 min at room temperature. Fixed cells were washed with PBS, and stained with 4,6-diamidino-2-phenylindole (DAPI, Sigma) solution for 10 min at room temperature. The cells were washed two more times with PBS and analyzed via a fluorescent microscope.

DNA fragmentation assay. After treatment with dideoxypetrosynol A, cells were lysed in a buffer containing 10 mM Tris-HCl (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:isoamylalcohol (25:24:1, v/v/v) and analyzed electrophoretically on 1% agarose gel containing 0.1 μg/ml ethidium bromide (EtBr).

Flow cytometric analysis. After treatment with dideoxypetrosynol A, cells were trypsinized, washed with PBS, and fixed in 75% ethanol at 4˚C for 30 min. Prior to analyses, cells were again washed with PBS, suspended in cold propidium iodide (PI, Sigma) solution, and incubated at room temperature in the dark for 30 min. Flow cytometry analyses were performed on a FACScan flow cytometry system (Becton Dickinson, San Jose, CA).

RNA extraction and reverse transcription-PCR. Total RNA was isolated according to a previously published method (19). Single-strand cDNA was synthesized from 2 μg of total RNA using M-MLV reverse transcriptase (Gibco-BRL). The mRNAs were amplified by polymerase chain reaction (PCR) with indicated primers in Table I. Conditions for PCR reaction were 1x (94˚C for 3 min); 35x (94˚C for 45 sec; 58˚C for 45 sec; and 72˚C for 1 min) and 1x (72˚C for 10 min). Amplification products obtained by PCR were electrophoretically separated on 1% agarose gel and visualized by EtBr staining.

Gel electrophoresis and Western blot analysis. The cells were harvested, lysed, and protein concentrations were quantified using the Bio-Rad protein assay (Bio-Rad Lab., Hercules, CA), following the procedure described by the manufacturer. Western blot analysis was performed as described (20,21). Briefly, an equal amount of protein was subjected to electrophoresis on SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Schleicher & Schuell, Keene, NH) by electroblotting. Blots were probed with the desired

Table I. Oligonucleotides used in RT-PCR.

Name		Sequence of primers
COX-1	Sense	5'-TGC-CCA-GCT-CCT-GGC-CCG-CCG-CTT-3'
	Antisense	5'-GTG-CAT-CAA-CAC-AGG-CGC-CTC-TTC-3'
COX-2	Sense	5'-TTC-AAA-TGA-GAT-TGT-GGG-AAA-AT-3'
	Antisense	5'-AGA-TCA-TCT-CTG-CCT-GAG-TAT-CTT-3
hTERT	Sense	5'-AGC-CAG-TCT-CAC-CTT-CAA-CC-3'
	Antisense	5'-GTT-CTT-CCA-AAC-TTG-CTG-ATG-3'
TEP-1	Sense	5'-TCA-AGC-CAA-ACC-TGA-ATC-TGA-G-3'
	Antisense	5'-CCC-CGA-GTG-AAT-CTT-TCT-ACG-C-3'
hTR	Sense	5'-TCT-AAC-CCT-AAC-TGA-GAA-GGG-CGT-AG-3'
	Antisense	5'-GTT-TGC-TCT-AGA-ATG-AAC-GGT-GGA-AG-3'
c-myc	Sense	5'-AAG-ACT-CCA-GCG-CCT-TCT-CTC-3'
	Antisense	5'-GTT-TTC-CAA-CTC-CGG-GAT-CTG-3'
GAPDH	Sense	5'-CGG-AGT-CAA-CGG-ATT-TGG-TCG-TAT-3'
	Antisense	5'-AGC-CTT-CTC-CAT-GGT-GGT-GAA-GAC-3'

antibodies for 1 h, incubated with diluted enzyme-linked secondary antibodies and then visualized by the enhanced chemilumi-nescence (ECL) according to the recommended procedure (Amersham Corp., Arlington Heights, IL). The primary anti-bodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Peroxidase-labeled donkey anti-rabbit immunoglobulin and peroxidase-labeled sheep anti-mouse immunoglobulin were purchased from Amersham.

Determination of caspase activation. Caspase activity was determined by colorimetric assay using the caspase-3 and caspase-9 activation kits from Clontech Lab. (Palo Alto, CA) and R&D Systems (Minneapolis, MN), respectively, following the manufacturer's protocol. The chromophore used in both cases was *p*-nitroaniline (pNA), which was released from Asp-Glu-Val-Asp (DEVD)-pNA by caspase-3 and from Leu-Glu-His-Asp (LEHD)-pNA by caspase-9. The reaction was measured by changes in absorbance at 405 nm using the VERSAmax tunable microplate reader (Molecular Devices, Palo Alto, CA).

PGE_2 EIA analysis. After treating with dideoxypetrosynol A, the medium was removed and PGE_2 release by cells was measured. To measure the PGE_2 accumulation, enzyme immunoassay (EIA) was performed using a commercial kit (Cayman Chemicals, Ann Arbor, MI) according to the manufacturer's protocol. PGE_2 production was normalized with respect to the number of viable cells present in the particular culture.

Telomerase activity assay. Telomerase activity was measured using a PCR-based telomeric repeat amplification protocol (TRAP) enzyme-linked immunosorbent assay (ELISA) kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's description. In brief, cells were treated with dideoxypetrosynol A, harvested and ~1x10^6 cells were lysed in 200 μl lysis reagent and incubated on ice for 30 min. For the TRAP reaction, 2 μl of cell extract (containing 2 μg protein) was added to 25 μl of reaction mixture with the appropriate amount of sterile water to make a final volume of 50 ml. PCR was performed in a Mastercycler as follows: primer elongation (30 min, 25˚C), telomerase inactivation (5 min, 94˚C) and product amplification by the repeat of 30 cycles (94˚C for 30 sec, 50˚C for 30 sec, 72˚C for 90 sec). Hybridization and the ELISA reaction were carried out following the manufacturer's instructions.

Results

Inhibition of the viability and induction of apoptosis by dideoxypetrosynol A. To evaluate the effects of dideoxy-petrosynol A on cell proliferation of a human leukemia cell line, we initially assessed the effect on the growth of U937 cells. As shown in Fig. 1B, cell growth was inhibited by dideoxypetrosynol A in a dose-dependent manner. In order to determine whether the growth inhibition by dideoxy-petrosynol A was associated with the induction of apoptosis, DAPI staining was performed. As shown in Fig. 2A, the control cells displayed intact nuclear structure, while nuclei with chromatin condensation and formation of apoptotic bodies, a characteristic of apoptosis, were seen in cells cultured with dideoxypetrosynol A in a dose-dependent fashion. We also analyzed whether DNA fragmentation, another hallmark of apoptosis, was induced by dideoxypetrosynol A in U937 cells. Following agarose gel electrophoresis of U937 cells treated with 0.5 μg/ml and 1.0 μg/ml dideoxypetrosynol A for 48 h, a typical ladder pattern of internucleosomal fragmentation was observed (Fig. 2B). We next analyzed the amount of sub-G1 DNA, which contained less DNA than G1 cells, to

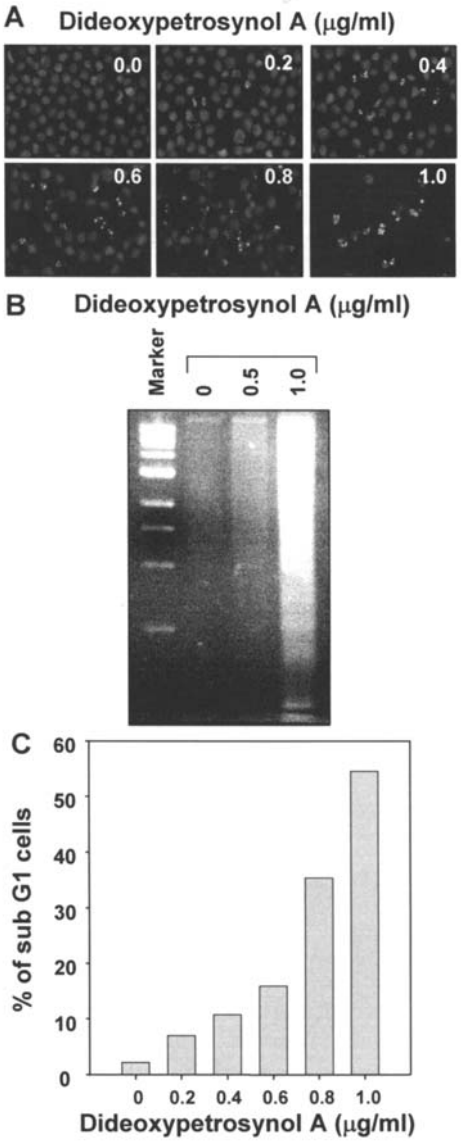

Figure 2. Induction of apoptosis by dideoxypetrosynol A in U937 cells. (A) Cells were cultured for 48 h with vehicle alone or dideoxypetrosynol A before cells were fixed and stained with DAPI. After 10-min incubation at room temperature, stained nuclei were then observed under a fluorescent microscope using a blue filter. Magnification x400. (B) To analyze fragmentation of genomic DNA, cells were treated for 48 h with the indicated concentrations of dideoxypetrosynol A. Fragmented DNA was extracted and analyzed on 1% agarose gel. (C) Cells treated for 48 h with increasing concentration of dideoxypetrosynol A. Then the cells were collected and stained with PI for flow cytometry analysis. The fraction of apoptotic sub-G1 cells is indicated. Data are presented as the mean values obtained from two independent experiments.

quantify the degree of dead cells. Flow cytometric analysis indicated that dideoxypetrosynol A treatment resulted in a markedly increased accumulation of sub-G1 phase in a dose-dependent manner (Fig. 2C). Taken together, these results demonstrated that the cytotoxic effects observed in response to dideoxypetrosynol A is associated with the induction of apoptotic cell death.

Induction of Bax protein and activation of caspases by dideoxypetrosynol A. In light of the importance of the expression of the Bcl-2 protein family, we evaluated the levels of Bax, Bcl-2 and Bcl-xL in dideoxypetrosynol A-treated U937 cells.

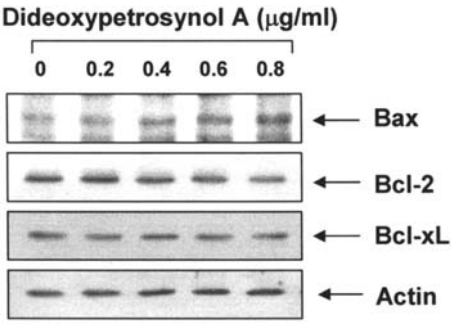

Figure 3. Effects of dideoxypetrosynol A on Bcl-2 family expression in U937 cells. After 48-h incubation with dideoxypetrosynol A, cells were lysed and then cellular proteins were separated by 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the anti-Bax, anti-Bcl-2 and anti-Bcl-xL antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control.

Western blot analysis revealed that the anti-apoptotic Bcl-2 and Bcl-xL levels remained virtually unchanged in response to dideoxypetrosynol A (Fig. 3), whereas pro-apoptotic Bax expression was increased by dideoxypetrosynol A treatment, suggesting dideoxypetrosynol A alters the Bax:Bcl-2 and Bax:Bcl-xL ratio in U937 cells. To gain further insight into the mechanism by which dideoxypetrosynol A induces apoptosis, we examined the effects of dideoxypetrosynol A on caspase-3 and caspase-9 protein levels and their activity. As shown in Fig. 4A, dideoxypetrosynol A-induced apoptosis of U937 cells was associated with a decreased expression of the pro-caspase-3 and pro-caspase-9 protein; however, the active subunits of both caspases were not observed. To quantify the activities of caspase-3 and caspase-9, lysates equalized for protein from cells treated with dideoxypetrosynol A were assayed for *in vitro* caspase-3 and caspase-9 activity using DEVD-pNA and LEHD-pNA, respectively, as fluorogenic substrates. As indicated in Fig. 4B, the results showed that dideoxypetrosynol A exposure markedly increased both caspase-3 and caspas-9 activity in a concentration-dependent manner. Furthermore, dideoxypetrosynol A induced a concomitant degradation of PARP protein and accumulation of the 85 kDa, which is a substrate protein of caspase-3. We also examined the effects of dideoxypetrosynol A on the family of anti-apoptotic inhibitory apoptosis proteins (IAPs), which bind to caspases and lead to inactivation of caspases. As shown in Fig. 4C, dideoxypetrosynol A markedly inhibited cIAP-1 and cIAP-2, but not XIAP in U937 cells.

Inhibition of COX-2 activity and PGE_2 production by dideoxypetrosynol A. We next determined whether dideoxypetrosynol A-induced anti-proliferative effect of U937 cells was connected with a reduced activity of COXs. The RT-PCR and Western blot analyses showed a significant decrease in COX-2 mRNA and protein expression after dideoxypetrosynol A treatment but, dideoxypetrosynol A was ineffective on COX-1 expression, respectively (Fig. 5A and B). To confirm that PGE_2 production was associated with the catalytic activity of COX-2 isoform, U937 cells were cultured in the presence of dideoxypetrosynol A, and PGE_2 levels were measured. As

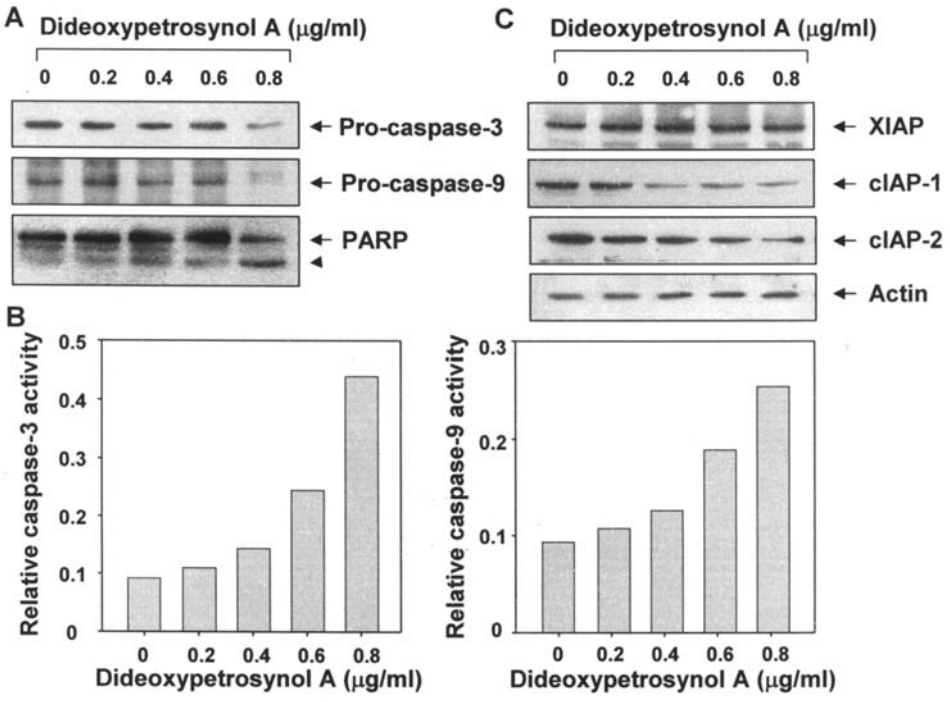

Figure 4. Activation of caspase-3 and -9, and degradation of PARP protein, and inhibition of IAP family proteins by dideoxypetrosynol A in U937 cells. (A and C) After 48-h incubation with dideoxypetrosynol A, the cells were lysed and then cellular proteins were separated by 8% or 12% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with indicated antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control. (B) Cell lysates from cells treated with dideoxypetrosynol A for 48 h were assayed for *in vitro* caspase-3 and -9 activity using DEVD-pNA and LEHD-pNA-pNA, respectively, as substrates at 37˚C for 1 h. The released fluorescent products were measured. Data are presented as the mean values obtained from two independent experiments.

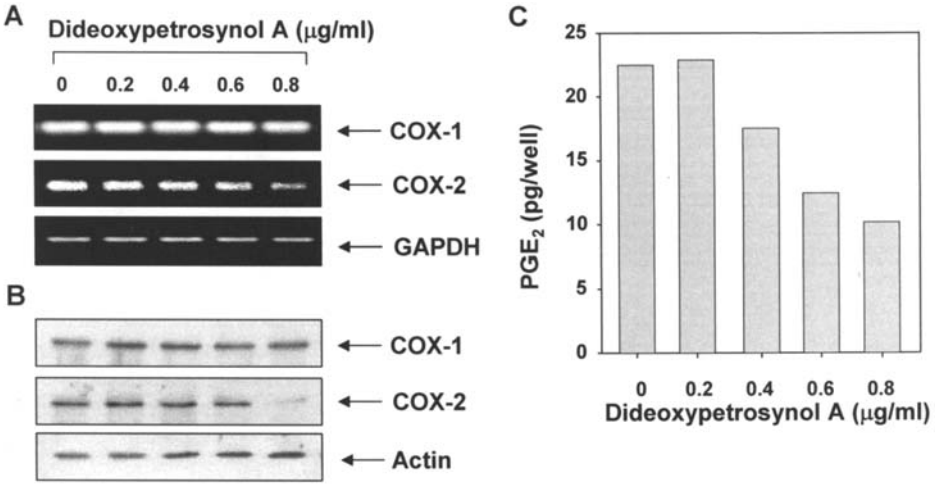

Figure 5. Down-regulation of COX-2 expression and inhibition of PGE_2 production by dideoxypetrosynol A in U937 cells. (A) After 48-h incubation with dideoxypetrosynol A, total RNAs were isolated and reverse-transcribed. The resulting cDNAs were subjected to PCR with COX-1 and COX-2 primers and the reaction products were subjected to electrophoresis in a 1% agarose gel and visualized by EtBr staining. GAPDH was used as an internal control. (B) Cells were treated with the indicated concentrations of dideoxypetrosynol A for 48 h and collected. The cells were lysed and then cellular proteins were separated by 10% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the antibodies against COX-1 and COX-2. Proteins were visualized using an ECL detection system. Actin was used as an internal control. (C) Cells were treated with the indicated concentrations of dideoxypetrosynol A for 48 h and collected. The PGE_2 accumulation in the medium was determined by an EIA kit as described in Materials and methods. Data represent the relative mean values of two independent experiments.

shown in Fig. 5C, the production of PGE_2 was significantly decreased after dideoxypetrosynol A treatment in a concentration-dependent manner, which was well correlated with down-regulation of COX-2 expression.

Inhibition of telomerase activity and hTER expression by dideoxypetrosynol A. To examine the effects of dideoxypetrosynol A on telomerase activity, U937 cells were cultured with various concentrations of dideoxypetrosynol A for 48 h

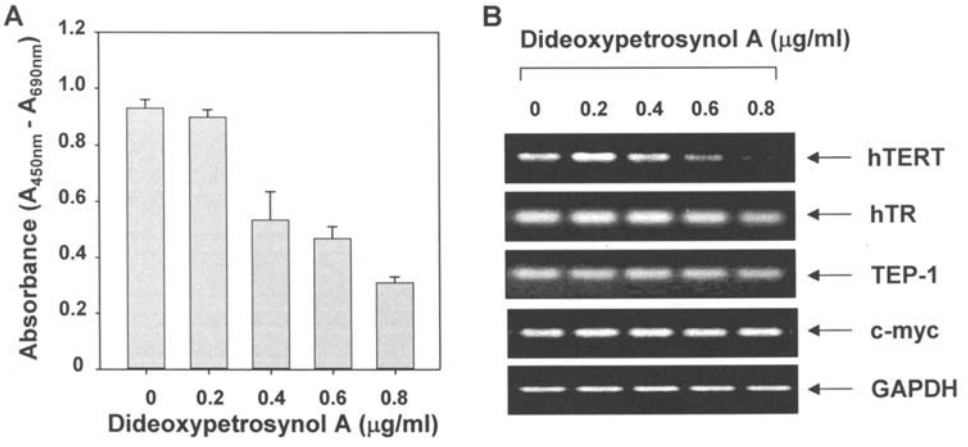

Figure 6. Inhibition of telomerase activity by dideoxypetrosynol A treatment in U937 cells. (A) After 48-h incubation with dideoxypetrosynol A, telomerase activity of U937 cells were measured using a TRAP-ELISA kit as described in Materials and methods. For one sample, $2x10^5$ cells were lysed, and 1/100 was used in the assay. Data represent the relative mean values ± SE of three independent experiments. (B) After 48-h incubation with dideoxypetrosynol A, total RNA was isolated and RT-PCR was performed using indicated primers. The amplified PCR products were run in a 1% agarose gel and visualized by EtBr staining. GAPDH was used as a house-keeping control gene.

and telomerase activity was measured by TRAP-ELISA. As shown in Fig. 6A, telomerase activity was reduced by dideoxypetrosynol A treatment in a dose-dependent manner. Of the components comprising telomerase, hTERT is a critical determinant of the enzyme activity of telomerase. Therefore, we focused on the effects of dideoxypetrosynol A on hTERT mRNA levels. We used the RT-PCR method to examine the expression of hTERT, hTR, TEP-1, and c-myc mRNA. We found that hTERT mRNA levels were decreased but not in hTR, TEP-1 and c-myc mRNA levels and its down-regulation was concentration-dependent and could be observed at 0.6 μg/ml dideoxypetrosynol A (Fig. 6B). The results suggest that the repression of telomerase activity by dideoxypet-rosynol A was associated with down-regulation of hTERT mRNA levels.

Discussion

The present results clearly demonstrate that dideoxypetrosynol A, a polyacetylene from the marine sponge *Petrosia* sp., induces apoptosis in U937 cells, which appears to account for its anti-proliferating activity. The induction of apoptosis by dideoxypetrosynol A conformed to the characteristic morphological changes, DNA fragmentation and increase of sub-G1 cells (Fig. 2). The regulation of apoptosis is a complex process and involves a number of gene products including Bcl-2 protein family. It has been reported that Bcl-2 protects against multiple signals that lead to cell death, indicating that Bcl-2 regulates a common cell death pathway and functions at a point where various signals converge (22,23). Bcl-2 acts to inhibit cytochrome *c* translocation from mitochodria to cytoplasm, thereby blocking the caspase activation step of the apoptotic process (23,24). Thus, it has been suggested that the ratio between the level of pro-apoptotic Bax protein and that of the anti-apoptotic factor Bcl-2 protein determines whether a cell responds to an apoptotic signal (25). In our study, there was a concentration-dependent increase of Bax protein levels in U937 cells treated with dideoxypetrosynol A, but the levels of Bcl-2 members such as Bcl-2 and Bcl-xL remained unchanged, resulting in an increase in the ratio of Bax/Bcl-2 and Bcl-xL (Fig. 3).

The caspase family also plays an important role in driving apoptosis and the key components of the biochemical pathways of caspase activation have been recently elucidated (26). They are synthesized initially as single polypeptide chains representing latent precursors that undergo proteolytic processing at specific residues to produce subunits that form the active heterotetrameric protease. IAP family 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 (24,27). Further studies have shown that exposure of U937 cells to dideoxypetrosynol A caused a proteolytic activation of caspase-3 and caspase-9 (Fig. 4), and a down-regulation of cIAP-1 and cIAP-2 (Fig. 3). Activated caspases induce a limited proteolysis in a number of cellular proteins, which are degraded as a consequence of apoptosis by the caspase family and have been used as a marker of chemotherapy-induced apoptosis (28,29). Here, we examined whether PARP protein, a substrate of caspase-3 (30), was cleaved in cells treated with dideoxypetrosynol A. As expected, PARP protein was clearly degraded in a dose-dependent manner, again correlating with an activation of capases during apoptosis by dideoxypetrosynol A treatment. Taken together, our data indicated that the apoptotic effects of dideoxypetrosynol A on U937 cells are associated with up-regulation of the pro-apoptotic Bax, activation of caspases and the alteration of IAP expression.

The conversion of arachidonic acid to PGs is catalyzed by two isoenzymes, COX-1 and COX-2. COX-1 is expressed in most tissues that generate PGs during their normal physiological functions, and its expression does not fluctuate in response to stimuli (31). However, COX-2 expression can be induced by various agents, including inflammatory cytokines, mitogens, reactive oxygen intermediates and many other tumor promoters (32,33). In a number of experimental studies, induction of COX-2 has been shown to promote cell growth, inhibit apoptosis and enhance cell motility (2,3,6). It has also

been reported that the overexpression of COX-2 in normal cells leads to increased carcinogenesis, metastatic potential and angiogenesis and increased expression of COX-2 has been reported in various types of cancer (4). Although the role of COX-2 in tumor development and progression has not been fully elucidated, the development of specific COX-2 inhibitors promise to be an effective approach in the prevention and treatment of cancer (2,34). In the present study, we observed that dideoxypetrosynol A markedly inhibited COX-2 mRNA and protein expression in U937 cells, however, the levels of COX-1 remained unaltered (Fig. 5A and B). Additionally, the amount of PGE_2 produced by U937 cells was decreased with the increase of dideoxypetrosynol A concentration (Fig. 5C). The data suggested that the inhibition of the COX-2 expression and PGE_2 production is consistent with the results that dideoxypetrosynol A inhibited the growth and induced apoptosis.

It has been suggested that telomerase might be an important factor in the proliferative capacity that is important for both cellular immortalization and carcinogenesis. The important role of telomerase in replicative senescence and the specific expression of this enzyme in most cancers raise the potential use of telomerase inhibition for cancer therapy. The majority of immortal and cancer cells have an indefinite proliferative capacity and maintain their telomere length by upregulating telomerase activity (35,36). Bodnar *et al* (36) reported that ectopic expression of telomerase has been shown to extend the life-span of several types of normal human cells (36). Moreover, ectopic expression of hTERT in combination with oncogenes has been shown to be sufficient to convert normal human epithelial and fibroblast cells to tumor cells (37). According to the telomere hypothesis for replicative senescence, inhibition of telomerase in the malignant cells is anticipated to result in the erosion of telomeres and ultimately leading to growth arrest, senescence or cell death. Indeed, inhibition of telomerase has been shown to limit the growth of human cancer cells in culture (38,39), supporting the potential use of telomerase inhibition for cancer therapy. As indicated in Fig. 6, dideoxypetrosynol A induced a concentration-dependent inhibition of telomerase activity and hTERT expression without altering the expression of hTR and TEP-1. Telomerase activity could be regulated by c-myc through controlling the expression of hTERT gene (38,40). The ability of c-myc to function as a transcription factor has been shown to depend upon its dimerization with the protein Max (41). However, in the present study, dideoxypetrosynol A treatment did not affect the c-myc levels (Fig. 4B). The results suggested that the repression of telomerase activity by dideoxypetrosynol A was associated with down-regulation of hTERT mRNA without alteration of c-myc expression.

In conclusion, our results suggest that dideoxypetrosynol A induces an inhibition of human leukemia U937 cell growth with an apoptosis induction. The apoptotic events of U937 cells by dideoxypetrosynol A were mediated by an increase in Bax expression and an activation of caspases. Dideoxypetrosynol A concomitantly causes a decrease of PGE_2 production and, at least in part, down-regulation of telomerase activity by decreasing the COX-2 and hTERT expressions. These novel phenomena have not been previously described and provide important new insights into the possible biological effects of dideoxypetrosynol A. Although further studies are needed, the present work suggests that the loss of COX-2 and telomerase activity may be a good surrogate biomarker for assessing anti-tumor activity of dideoxypetrosynol A.

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