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Arachidin-1, a Peanut Stilbenoid, Induces Programmed Cell Death in Human Leukemia HL-60 Cells

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The stilbenoids, arachidin-1 (Ara-1), arachidin-3, isopentadienylresveratrol, and resveratrol, have been isolated from germinating peanut kernels and characterized as antioxidant and anti-inflammatory agents. Resveratrol possesses anticancer activity, and studies have indicated that it induces programmed cell death (PCD) in human leukemia HL-60 cells. In this study, the anticancer activity of these stilbenoids was determined in HL-60 cells. Ara-1 had the highest efficacy in inducing PCD in HL-60 cells, with an approximately 4-fold lower EC_{50} than resveratrol. Ara-1 treatment caused mitochondrial membrane damage, activation of caspases, and nuclear translocation of apoptosis-inducing factor, resulting in chromosome degradation and cell death. Therefore, Ara-1 induces PCD in HL-60 cells through caspase-dependent and caspase-independent pathways. Ara-1 demonstrates its efficacy as an anticancer agent by inducing caspase-independent cell death, which is an alternative death pathway of cancer cells with mutations in key apoptotic genes. These findings indicate the merits of screening other peanut stilbenoids for anticancer activity.

KEYWORDS: Arachidin-1; stilbenoids; anticancer; leukemia HL-60 cell; program cell death; apoptosis-inducing factor

INTRODUCTION

Stilbenoids exist widely as natural phytoalexins produced by plants such as grapes, berries, and peanuts in response to microbial infection. Resveratrol and its derivatives, arachidin-1 (Ara-1), arachidin-3 (Ara-3), and isopentadienylresveratrol (IPD), are stilbenoids that have been isolated by various research groups from peanut kernels following various treatments (*1*−*8*). Among these peanut stilbenoids, the biological functions of resveratrol have been studied extensively, and it was found to be a chemopreventive phytochemical for cardiovascular disease and cancer. It also exhibits potent bioactivities as an antioxidant, anticancer, and anti-inflammatory agent (*9*−*12*). The bioactivities of Ara-1, Ara-3, and IPD have not been extensively studied, with the exception of their reported antioxidant and anti-inflammatory activities (*7*, *13*). Anticancer activities of resveratrol have been studied in a variety of cancer cell systems, and it was found to effectively induce cancer cell death through different death pathways such as apoptosis and autophagy (*14*−*16*). The anticancer activity of the other peanut stilbenoids remains unknown.

Programmed cell death (PCD) is a basic biological process required for maintaining homeostasis in organisms. Apoptosis has long been known as a form of PCD distinguished from necrosis, which is an uncontrolled cell death pathway. Activation of caspases is the hallmark of apoptotic cell death. However, nonapoptotic PCD pathways have recently been identified as alternative mechanisms in response to cytotoxic agents or death stimuli to guard the organism against potentially harmful agents. Cell death induced by apoptosis-inducing factor (AIF) is indicative of nonapoptotic PCD (*17*−*19*). AIF is a metabolic enzyme imbedded in the inner mitochondrial membrane; however, upon initiation of death signals, it is proteolytically cleaved and translocated to the nucleus to mediate caspase-independent PCD. Nuclear AIF binds DNA and activates endonuclease, which results in cell death that is phenotypically similar to caspase-mediated apoptosis (*19*, *20*). Apoptosis may be mediated by either the extrinsic pathway (initiated by the Fas/Fas ligand system leading to caspase-8 activation) or the intrinsic pathway (initiated by depolarization of mitochondrial membranes leading to caspase-9 activation). The disruption of the mitochondrial membrane, an early event in intrinsic apoptosis, also causes the release of AIF from the mitochondria to nuclei (*18*−*20*). Resveratrol has been reported to induce Fas ligand-related apoptosis in human cancer cells including HL-60 leukemia cells (*14*, *15*) and to induce mitochondrion-mediated apoptosis in acute lymphoblastic leukemia cells (*16*). Previous studies on the anti-inflammatory activity

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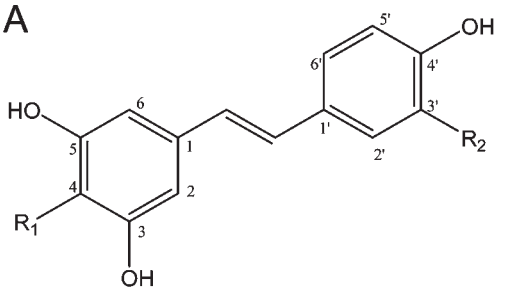

	R_1	R_2
Ara-1	C_5H_9[a]	OH
Ara-3	C_5H_9[a]	H
IPD	H	C_5H_9[b]
Resveratrol	H	H

[a]3-methyl-1-butenyl-

[b]3-methyl-1,3-butadienyl-

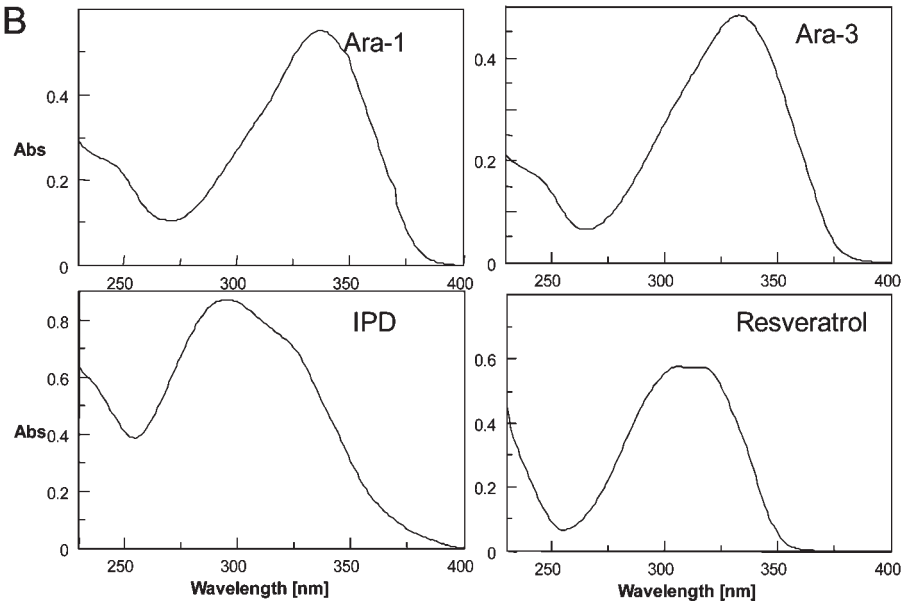

Figure 1. (**A**) Structures and (**B**) absorption spectra of Ara-1, Ara-3, IPD, and resveratrol. Resveratrol: *trans*-3,5,4′-trihydroxystilbene. Ara-1: *trans*-4-(3-methyl-1-butenyl)-3,5,3′,4′-tetrahydroxystilbene. Ara-3: *trans*-4-(3-methyl-1-butenyl)-3,5,4′-trihydroxystilbene. IPD: *trans*-3′-isopentadienyl-3,5,4′-trihydroxystilbene.

of Ara-1, Ara-3, and IPD have indicated that Ara-1 is more potent than Ara-3 and IPD and is as effective as resveratrol in the inhibition of lipopolysaccharide-induced up-regulation of PGE_2 and NO biosynthesis in mouse RAW 264.7 macrophage cells (*7*). A further mechanistic study has shown that Ara-1 and resveratrol modulate inflammatory responses by affecting the levels of transcription factors NF-κB and C/EBPδ (*13*). In the current study, resveratrol, Ara-1, Ara-3, and IPD were isolated from germinating peanut kernels, and their anticancer activities were assessed in leukemia HL-60 cells. The potency of Ara-1 as an anticancer agent and its efficacy-associated contribution to the induction of PCD, including apoptosis and caspase-independent cell death, were further determined.

MATERIALS AND METHODS

Purification and Analysis of the Peanut Stilbenoids. Stilbenoids were isolated from germinating peanut kernels (*Arachis hypogaea* L.) with procedures modified from a previous report (*7*). Briefly, peanut sprout powder (0.1 g) was dissolved in 60% ethanol (5 mL), sonicated briefly, and shaken vigorously at 25 °C for 30 min. The suspensions were centrifuged and filtered through 0.45 μm filter membranes. The filtrate was subjected to separation by HPLC (Chrom Tech, Inc., Apple Valley, MN) using a C-18 column (250 × 10 mm, DiscoveryBIO wide pore C18, Supelco Inc.; St. Louis, MO) eluted with 60% methanol at a flow rate of 3 mL/min. The elution profile was monitored by absorbance at 340 nm. For characterization and confirmation of the isolated compounds, the UV spectra of these constituents were recorded using a JASCO-V550 spectrophotometer, and their chemical structures were elucidated on the basis of 1-D NMR spectra (Bruker AMX-400 and Avance 500 FT-NMR spectrometers). The Mass spectra were analyzed by the Agilent 6510 Quadrupole Time-of-Flight LC/MS instrument equipped with a GRACE VYDAC 218MS, C18 column, 5 μm and 150 × 2.1 mm (Deerfield, IL).

Cell Culture and Compound Treatment. Human leukemia HL-60 cells were cultivated in RPMI-1640 medium supplemented with 10% fetal calf serum, 100 μg/mL streptomycin, 100 U/mL penicillin, 250 ng/mL amphotericin, 2 mM L-glutamine, and nonessential amino acids. Peripheral blood mononuclear cells (PBMCs) were obtained from healthy donors. The blood was centrifuged at 800*g* for 10 min at 4 °C to remove the plasma. Buffy coats were collected, suspended in phosphate-buffered saline (PBS), and then centrifuged in a Ficoll-Paque PLUS gradient (Amersham Pharmacia Biotech; Uppsala, Sweden) at 350*g* for 30 min. PBMCs were collected, washed with PBS, and cultured in RPMI-1640 medium at 37 °C under 5% humidified CO_2. Acid phosphatase activity assay (ACP assay) and trypan blue exclusion assay were used to measure overall cell viability and viable cell number. In the ACP assay, HL-60 cells (3×10^3 cells in 200 μL medium) were cultivated in 96-well plates for 16 h before the addition of a test compound. At the end of the treatment, the medium was aspirated, cells were washed with PBS, and incubated with 10 mM *p*-nitrophenyl phosphate containing 0.1 M sodium acetate and 0.1% Triton X-100. The reaction was terminated by adding NaOH solution, and the absorbance at 410 nm was measured using the SpectraMax M5 Microplate Reader (Molecular Devices; Sunnyvale, CA). The median

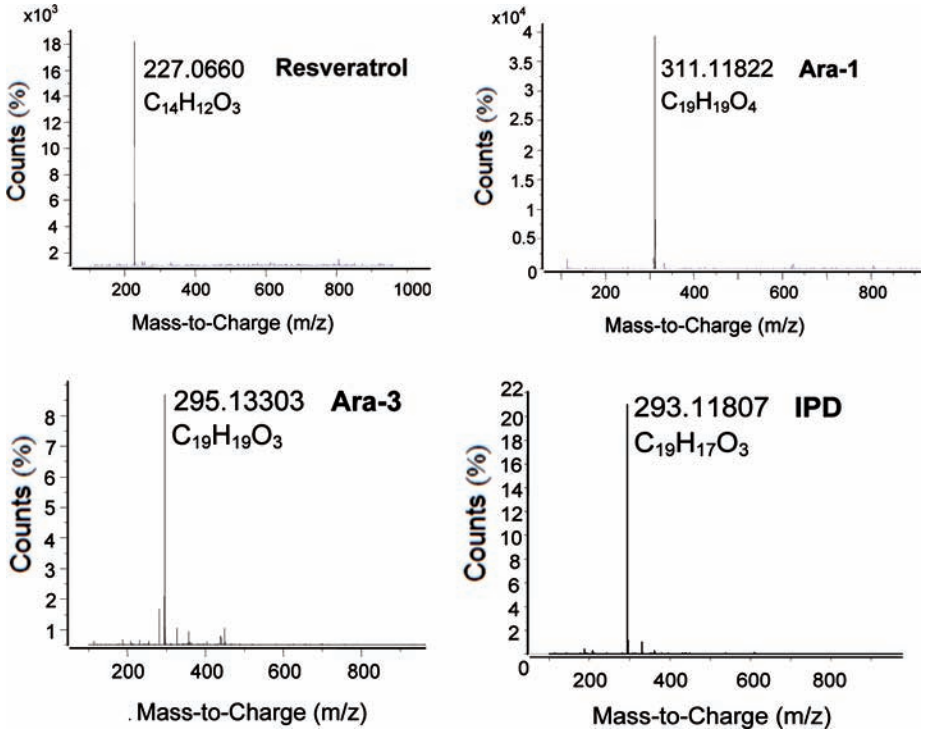

Figure 2. Mass spectra of resveratrol, Ara-1, Ara-3, and IPD.

effective concentration (EC_{50}), the concentration resulting in a 50% decrease in cell viability, was determined as previously described (*21*). In the trypan blue exclusion assay, the cells (5×10^4 cells in 3 mL of medium) were cultivated in 6-cm Petri dishes and treated with Ara-1. At the indicated time, 10 μL cell suspensions were mixed with 10 μL of trypan blue solution (0.4%). The number of unstained (viable) cells and stained (dead) cells was counted under a microscope. Each assay was done in triplicate, and at least 3 independent experiments were performed.

Analysis of Apoptotic Events by Flow Cytometry. HL-60 cells (5×10^4 cells in 3 mL medium) were cultivated in 6-cm Petri dishes. Time-dependent effects of Ara-1 were performed by treatment of the cells with 5 μM Ara-1, while concentration-dependent effects were studied using Ara-1 at concentrations from 1 μM to 4 μM. Mitochondrial injury was measured at 2 to 8 h after Ara-1 treatment. The cells were incubated with 100 nM $DiOC_6(3)$ in the dark for 1 h and subjected to flow cytometry using an Epics XL-MCL flow cytometer (Beckman Coulter Inc.; Fullerton, CA). Chromosomal degradation was assessed at 16, 24, and 48 h after the addition of Ara-1. The cells were washed with PBS, fixed in 70% ethanol, collected by centrifugation, resuspended in 0.5 mL of PBS containing 10 mg/mL RNase A, and incubated at 37 °C for 30 min. PI solution (0.5 mL) was then added, and the mixture was allowed to stand in the dark at room temperature. The cells were then subjected to flow cytometry. Data from at least 1×10^4 cells were collected and analyzed by multicycle software for cell cycle distribution and sub G1 population (*22*). The sub G1 population represents cells with degraded chromosomes. Caspase activities were measured using reagent kits for caspase-3 (DEVD-ase), caspase-8 (IETD-ase), and caspase-9 (LEHD-ase) (OncoImmunin Inc.; Gaithersburg, MD). The protocols suggested in the user manual were followed. To study the influence of caspase-family inhibitor Z-VAD-FMK (BioVision Research Products, Mountain View, CA), the inhibitor was added 3 h prior to the addition of Ara-1.

Protein Extraction and Western Blot Analysis. HL-60 cells (1×10^6 cells in 10 mL medium) were cultured in 10-cm Petri dishes, treated with Ara-1 for 24 h, and then collected by centrifugation. Protein extracts of nuclear and cytosolic fractions were prepared. The cells were first suspended in 400 μL of cold hypotonic buffer [10 mM HEPES (pH 7.8), 10 mM KCl, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.3 mg/mL leupeptin, and 0.1 mM PMSF] with the addition of 25 μL of 10% NP-40 and mixed vigorously on an ice bath. After a 20-min incubation, the solution was centrifuged at 4 °C at 10,000*g* for 5 min, and the supernatant was collected as the cytosolic fraction. The pellets containing nuclei were then resuspended in 100 μL of cold hypertonic buffer [50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, and 10% glycerol]. The suspension was sonicated twice for 10 s and centrifuged at 4 °C at 16,000*g* for 15 min, and the supernatants containing the nuclear proteins were collected and stored at −80 °C. The lysates were subjected to the BCA protein assay (Pierce; Rockford, IL) for the quantification of protein concentration. Aliquots of the lysates (50 μg of protein) were boiled with sample buffer for 5 min and resolved by 12% SDS−PAGE. The proteins were transferred to a nitrocellulose membrane and blocked in TBST buffer [10 mM Tris (pH 7.5), 100 mM NaCl, and 0.05% Tween 20] containing 5% skim milk. The membrane was incubated with specific primary antibodies and then incubated with the appropriate HRP-conjugated secondary antibody. Actin and proliferating cell nuclear antigen protein (PCNA) were used as protein loading controls. Monoclonal antibodies for AIF, actin, and PCNA were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Protein bands were visualized using an ECL chemiluminescence kit (Life Science Products; Boston, MA), and the gel images were analyzed by the Fujifilm LAS-4000 Imaging System (FUJIFILM Life Science, JAPAN).

RESULTS

Purification and Characterization of Peanut Stilbenoids. Four stilbene derivatives, resveratrol, Ara-1, Ara-3, and IPD, were isolated from germinating peanut kernels and identified in accordance with the 1-D NMR spectral data report previously (*7*, *8*). Ara-1, Ara-3, and IPD are derivatives of resveratrol with the derivatization of a hydroxyl moiety at C-3′ in Ara-1, an isopentadienyl moiety at C-4 in IPD, and an isopentenyl moiety at C-4 in Ara-1 and Ara-3 (**Figure 1A**). On HPLC, the compounds were eluted with retention times of 5.7 min (resveratrol), 10 min (Ara-1), 13 min (Ara-3), and 14 min (IPD). The characteristic UV absorption spectra (**Figure 1B**) with λ_{max} in 305 nm (resveratrol), 337 nm (Ara-1), 333 nm (Ara-3), and 295 nm (IPD) were checked routinely to monitor the purity and stability of the compounds. The LC/MS spectroscopy showed $[M - H]^-$ ion peaks at m/z 227.0660 for resveratrol, m/z 311.11822 for Ara-1, m/z 295.13303 for Ara-3, and m/z 293.11807 for IPD, indicating their molecular formula of $C_{14}H_{12}O_3$, $C_{19}H_{19}O_4$, $C_{19}H_{19}O_3$, and $C_{19}H_{17}O_3$ (**Figure 2**), respectively.

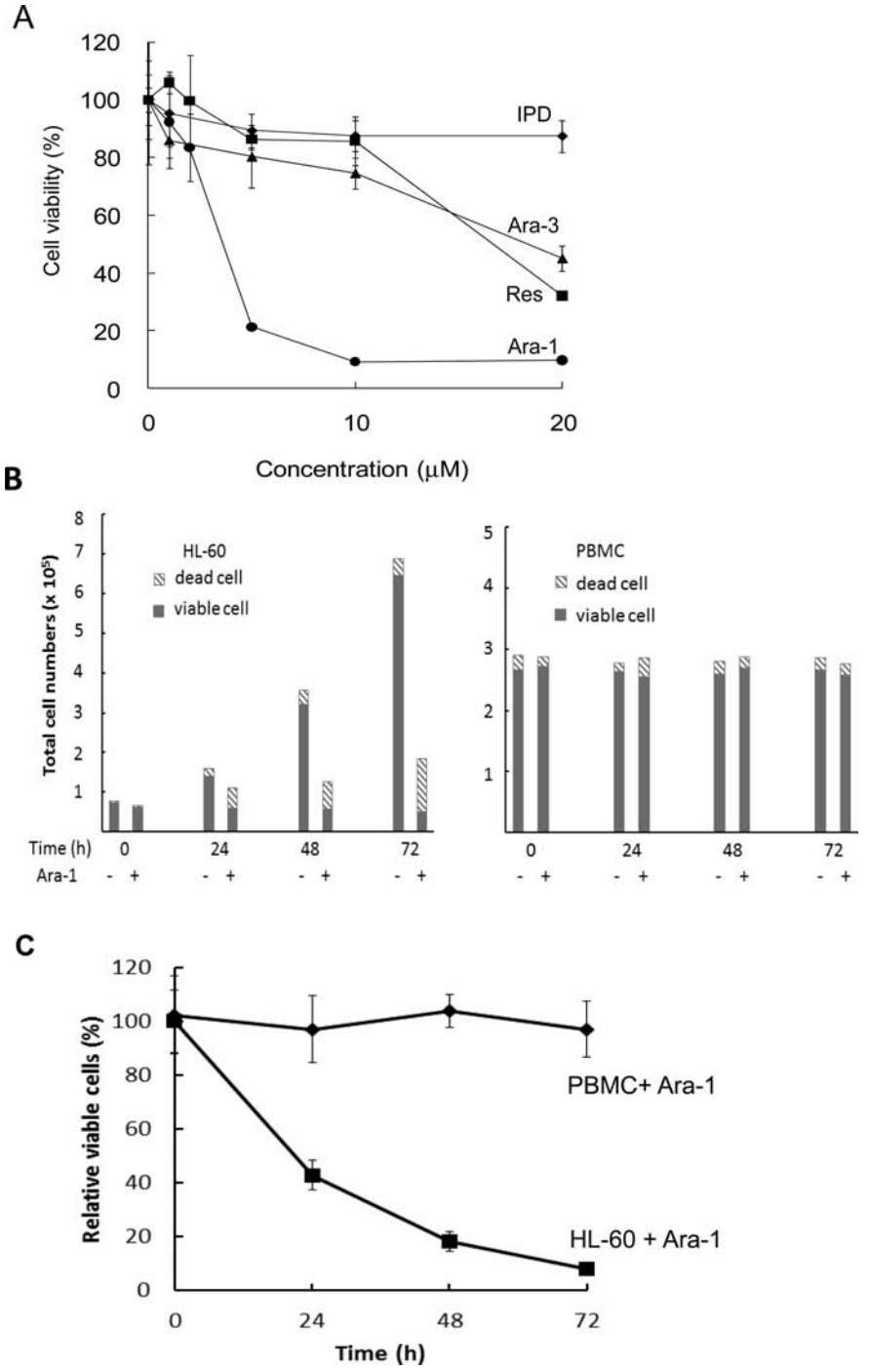

Figure 3. Growth inhibition and cell death of HL-60 cancer cells induced by peanut stilbenoids. (**A**) The cells were treated with Ara-1, Ara-3, IPD, and resveratrol (Res) for 72 h, and cell viability was measured. (**B**) PBMCs and HL-60 cells were treated with 5 μM Ara-1, cultured for the indicated time, and then subjected to trypan blue staining for counting viable cells and dead cells. (**C**) Relative viable cells (the percentage of viable cell number of the Ara-1-treated group relative to that of the untreated group) at each time point were calculated from the data in **B**.

Anticancer Effects of Peanut Stilbenoids. Over a concentration range of 0 to 20 μM, Ara-1, Ara-3, and resveratrol showed concentration-dependent growth inhibitory effects on HL-60 cells (**Figure 3A**) with EC_{50} values of 4.2 μM, 18.9 μM, and 17.6 μM, respectively. IPD showed little effect on HL-60 cells; at 20 μM, IPD resulted in only 10% decreases in cell viability. This research further studied Ara-1, the most potent cytotoxic compound, for its cytotoxic mechanism. The results of the trypan blue assay (**Figure 3B**) indicated that treatment with Ara-1 led to growth inhibition in HL-60 cells as reflected by differences in total cell numbers between the Ara-1-treated group and the control group. In addition to growth inhibition, Ara-1 also caused cell death; thus, relative viable cells declined dramatically (**Figure 3C**). However, Ara-1 exerted no effect on nonproliferating human PBMCs. (**Figure 3B** and **C**). Under the cultural conditions, Ara-1-treated PBMCs underwent spontaneous apoptosis at the same rate as the untreated PBMCs. The growth inhibition by Ara-1 could be due to cell cycle blockade at the G0/G1 cell cycle phase. **Figure 4B** shows 20% increases in G0/G1 cell population concomitant with 28% decreases in the S-phase population after 24 h of treatment. Flow cytometric data (**Figure 4A**) also revealed that Ara-1 treatment caused chromosome

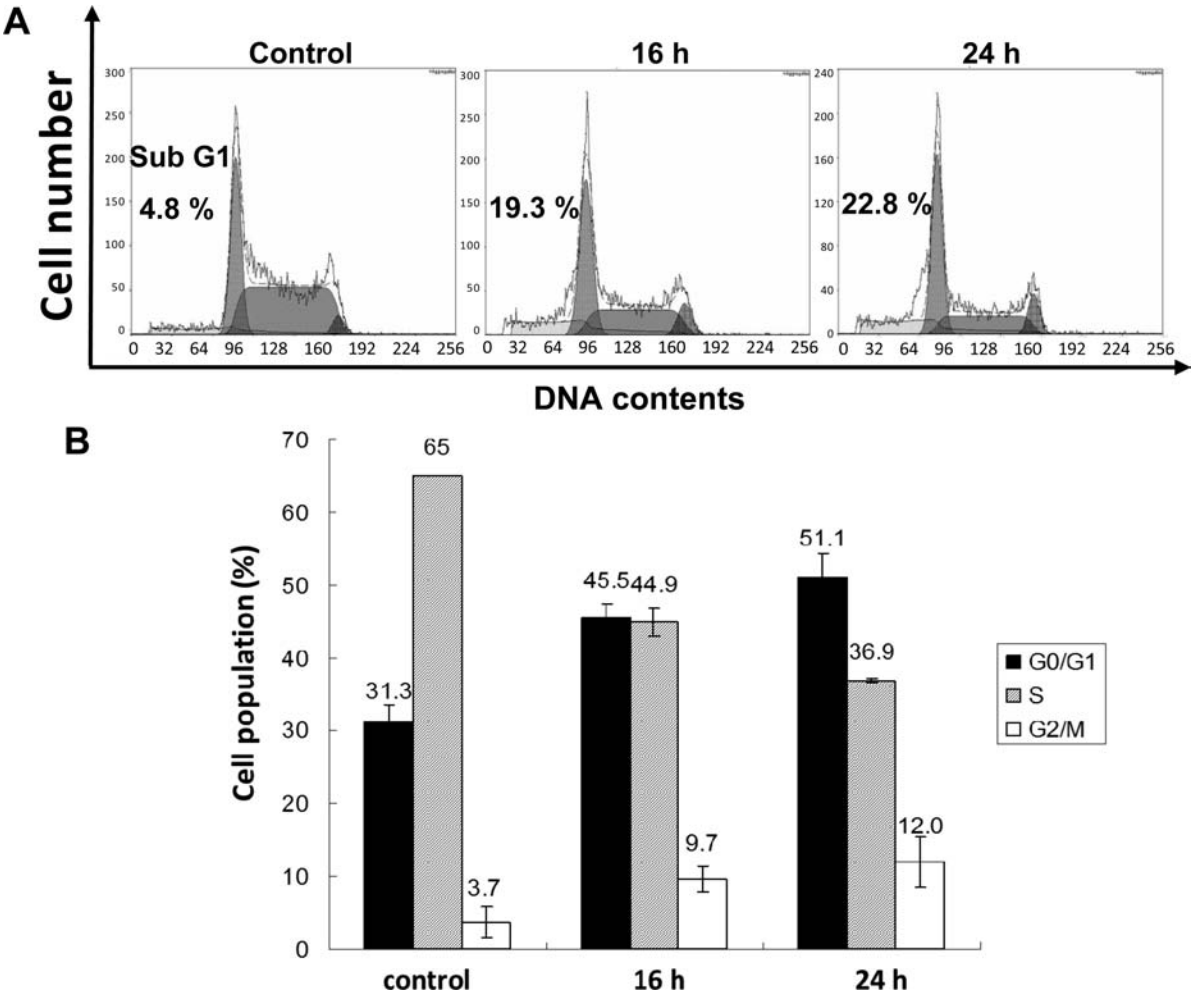

Figure 4. Cell-cycle arrest and chromosomal damage induced by Ara-1. (**A**) HL-60 cells were treated with 5 μM Ara-1 and subjected to DNA contents analysis. The percentage shown in each graph indicates the cells with chromosomal damage and shown as the sub G1 population. (**B**) Quantification of cell cycle distribution of the result in **A**. Results derived from 3 independent experiments are shown.

degradation (shown as sub G1 cell populations), resulting in cell death.

Ara-1 Induces Mitochondrion-Mediated Apoptosis. To study further the pathways involved in Ara-1-induced cell death, the current research measured mitochondrial membrane integrity, detecting mitochondrial membrane injury as an early event in Ara-1-treated HL-60 cells. **Figure 5A** shows that approximately 10% and 30% of the cells that lost mitochondrial membrane integrity were detected at 4 and 8 h. Contrarily, mitochondrial membrane injury went undetected in PBMCs (Supporting Information, Figure 1). Ara-1-caused apoptosis in HL-60 cells was evidenced by the activation of caspase-9 and caspase-3. The results in **Figure 5B** indicate that Ara-1 caused concentration-dependent activation of the caspases. However, findings did not show the activation of caspase-8 (data not shown). To elucidate how caspase activities contributed to cell death, the current study investigated the influence of the caspase-family inhibitor (Z-VAD-FMK). Ara-1 at low concentration induced approximately 10% of the sub G1 cell population, which was eliminated by Z-VAD-FMK pretreatment. However, at 4 μM Ara-1, the sub G1 cell population reached 45%, and nearly one-half of the population was not eliminated by Z-VAD-FMK pretreatment. These results suggest that Ara-1-mediated cell death attributed to not only caspase activity but also to other death mechanisms.

Ara-1 Induced Nuclear Translocation of AIF. Caspase-independent PCD could result from AIF release from the mitochondria to the nuclei, thereby activating endonucleases. Analysis of the mitochondrial form of AIF (62 kDa) and the nuclear truncated form of AIF (57 kDa) contributed to this investigation as to whether Ara-1 induced the nuclear shuttling of AIF (*20*). **Figure 6** shows that Ara-1 exerted no effect on the level of cytosolic AIF; however, the level of nuclear AIF significantly increased. Nuclear translocation of AIF suggests the involvement of a caspase-independent death mechanism.

DISCUSSION

This study found that Ara-1 induces depolarization of the mitochondrial membrane and cell death, executed by caspase-dependent and caspase-independent pathways in HL-60 leukemia cells. In this cell line, Ara-1 was much more cytotoxic than the other three stilbenoids tested. In this study, resveratrol had an EC_{50} of 17.6 μM, close to the previously obtained literature value of 16 μM, where resveratrol induced the apoptosis of HL-60 cells via the extrinsic pathway (*14*). Another study reported that resveratrol at 50 μM induced the intrinsic apoptotic pathway in leukemia cells via depolarizing the mitochondrial membrane and activating caspase-9 (*16*). The results of this study indicate that Ara-1 (EC_{50} = 4.2 μM) is much more potent than resveratrol and induces cell death in HL-60 cells through only the intrinsic apoptotic pathway. This research monitored depolarization of mitochondrial membranes as an early irreversible step of PCD by $DiOC_6$ (3) staining. Although research has questioned the suitability

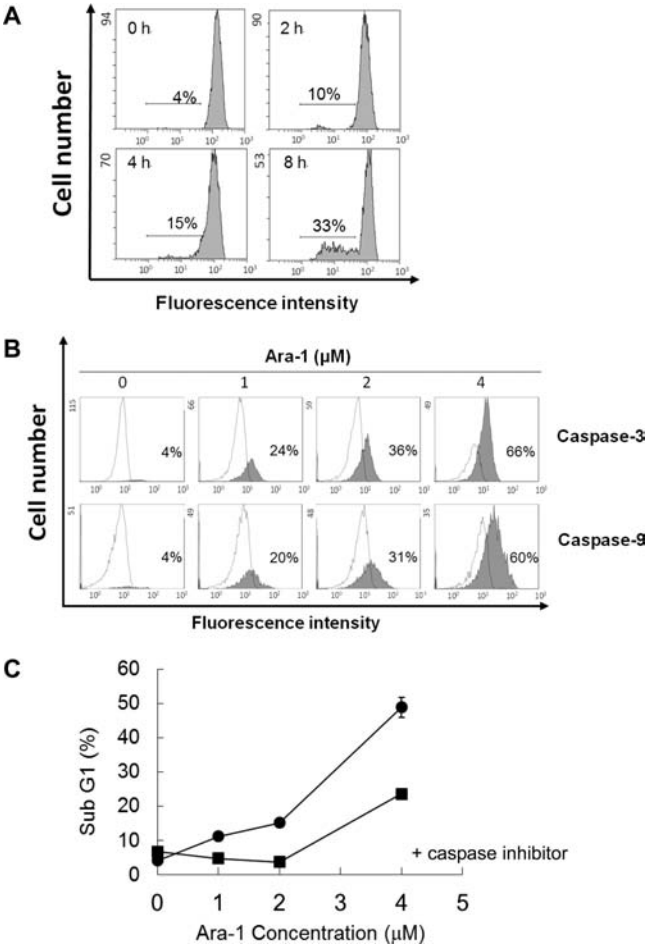

Figure 5. Mitochondrial disruption and caspase activation induced by Ara-1. (**A**) HL60 cells were treated with 5 μM Ara-1 for the indicated time, stained with $DiOC_6(3)$, and then analyzed by flow cytometry. The percentage shown in each graph indicates the cell population with disrupted mitochondrial membrane. (**B**) The cells were treated with Ara-1 for 24 h, and the activities of caspase-9 and caspase-3 were measured. The cells with activated caspase shown as the gray histogram are indicated in each graph. (**C**) The cells were treated with Ara-1 (●) or treated with the caspase-family inhibitor for 3 h, and then treated with Ara-1 (■). After 48 h, the cells were subjected to flow cytometry for sub G1 cell populations. Results derived from 3 independent experiments are shown.

of $DiOC_6$ for estimating mitochondrial membrane potential (*23*), the findings of caspase activation and the presence of chromosomal fragmentation ensure PCD induction. On the basis of the structure−activity relationship (SAR), the greater cytotoxicity of Ara-1, compared to resveratrol, may associate with the 4-isopentenyl moiety and the additional 3′-hydroxyl moiety. The isopentenyl group might increase the hydrophobicity of Ara-1, thereby facilitating compound interaction with the cell membrane, which could increase cell uptake. Second, the increase in hydrophobicity might also facilitate Ara-1 interactions with the mitochondrial membrane, thereby disrupting the outer membrane and triggering death signals. Studies have pointed out that the hydroxyl moieties of peanut stilbenoids are important determinants of antioxidant and antiproliferation activity (*7*). Many prenylated stilbenoids isolated from plants exhibit bioactivities (*24*−*26*). Among the isolates, mappain and vedelianin from *Macaranga* spp. are derivatives of Ara-1; they have 3′-hydroxyl and 4-isopentenyl moieties and an additional derivatization on the 5′ position. Both compounds exhibit potent anticancer activity (*24*, *25*). Nevertheless, how the 3′-hydroxyl group and

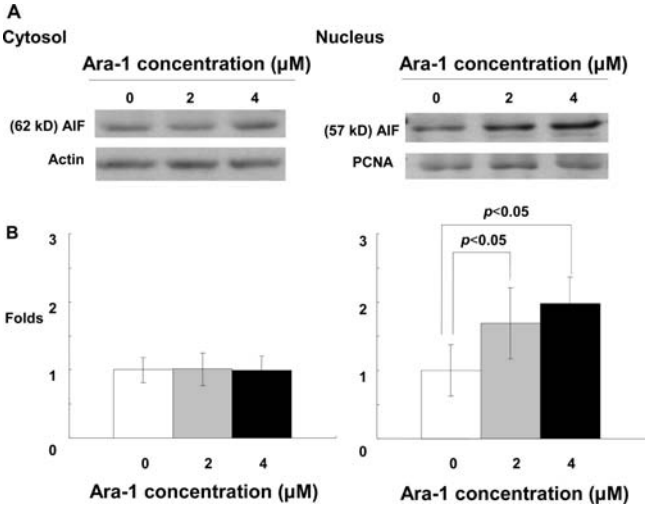

Figure 6. Activation and nuclear translocation of truncated AIF induced by Ara-1. (**A**) HL-60 cells were treated with Ara-1 for 24 h, and the levels of AIF in the cytosolic and nuclear fractions were analyzed. A representative blot from 3 independent experiments is shown. (**B**) Relative amount of AIF derived from the results in **A** and 2 additional experiments. The significance of the differences was analyzed by Student's *t*-test.

4-isopentenyl moieties play roles in anticancer activity is an interesting SAR issue needing further investigation. The proposed mechanism for Ara-1 in mitochondria depolarization is interesting, not only for developing a new anticancer drug but also for a new antiparasitic drug. For instance, the antiparasitic drug artemisinin functions through ROS production and depolarization of mitochondria in parasites (*27*).

Cancer is a complicated disease, and in many cases, the malignancy is caused by a gene mutation. Clinical studies have indicated that some chemotherapeutic agents are ineffective against tumor cells that have mutations in key apoptotic genes or those genes affecting caspase signaling (*28*). Agents that are capable of activating caspase-independent cell death pathways would be valuable in sensitizing these cells to death and eradicating tumor cells (*20*, *29*). Accordingly, the ability of Ara-1 to induce cell death by both activating the caspase cascade and a caspase-independent death pathway may explain its efficiency at triggering cell death. Therefore, Ara-1 has the potential to be developed as an anticancer agent, and the molecular mechanism involved in its toxicity warrants further investigation. The peanut plant is a rich source of stilbenoids (*2*). In addition to the stilbenoids investigated here, six novel stilbene derivatives (termed arahypins) were recently isolated from peanut seeds challenged by *Aspergillus caelatus* (*30*). The class of peanut stilbenoids consisting of structurally related compounds are potential resources for screening anticancer drugs.

ACKNOWLEDGMENT

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Supporting Information Available: Additional experimental results. This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Ingham, J. L. 3,5,4′-trihydroxystilbene as a phytoalexin from groundnuts (*Arachis hypogaea*). *Phytochemistry* **1976**, *15*, 1791−1793.
(2) Keen, N. T.; Ingham, J. L. New stilbene phytoalexins from American cultivars of *Arachis hypogaea*. *Phytochemistry* **1976**, *15*, 1794−1795.

(3) Aguamah, G. A.; Langcake, P.; Leworthy, D. P.; Page, J. A.; Pryce, R. J.; Strange, R. N. Two novel stilbene phytoalexins from *Arachis hypogaea*. *Phytochemistry* **1981**, *20*, 1381−1383.
(4) Wotton, H. R.; Strange, R. N. Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to *Aspergillus flavus*. *J. Gen. Microbiol.* **1985**, *131*, 487−494.
(5) Cooksey, C. J.; Garratt, P. J.; Richards, S. E.; Strange, R. N. A dienyl stilbene phytoalexin from *Arachis hypogaea*. *Phytochemistry* **1988**, *27*, 115−116.
(6) Sobolev, V. S.; Guo, B. Z.; Holbrook, C. C.; Lynch, R. E. Interrelationship of phytoalexin production and disease resistance in selected peanut genotypes. *J. Agric. Food Chem.* **2007**, *55*, 2195−2200.
(7) Chang, J. C.; Lai, Y. H.; Djoko, B.; Wu, P. L.; Liu, C. D.; Liu, Y. W.; Chiou, R. Y. Biosynthesis enhancement and antioxidant and anti-inflammatory activities of peanut (*Arachis hypogaea* L.) arachidin-1, arachidin-3, and isopentadienylresveratrol. *J. Agric. Food Chem.* **2006**, *54*, 10281−10287.
(8) Chen, R. S.; Wu, P. L.; Chiou, R. Y. Peanut roots as a source of resveratrol. *J. Agric. Food Chem.* **2002**, *50*, 1665−1667.
(9) Shankar, S.; Singh, G.; Srivastava, R. K. Chemoprevention by resveratrol: molecular mechanisms and therapeutic potential. *Front. Biosci.* **2007**, *12*, 4839−4854.
(10) Athar, M.; Back, J. H.; Tang, X.; Kim, K. H.; Kopelovich, L.; Bickers, D. R.; Kim, A. L. Resveratrol: a review of preclinical studies for human cancer prevention. *Toxicol. Appl. Pharmacol.* **2007**, *3*, 274−283.
(11) Udenigwe, C. C.; Ramprasath, V. R.; Aluko, R. E.; Jones, P. J. Potential of resveratrol in anticancer and anti-inflammatory therapy. *Nutr. Rev.* **2008**, *66*, 445−454.
(12) Nigro, P.; Bloise, E.; Turco, M. C.; Skhirtladze, A.; Montoro, P.; Pizza, C.; Piacente, S.; Belisario, M. A. Antiproliferative and pro-apoptotic activity of novel phenolic derivatives of resveratrol. *Life Sci.* **2007**, *81*, 873−883.
(13) Djoko, B.; Chiou, R. Y.; Shee, J. J.; Liu, Y. W. Characterization of immunological activities of peanut stilbenoids, arachidin-1, piceatannol, and resveratrol on lipopolysaccharide-induced inflammation of RAW 264.7 macrophages. *J. Agric. Food Chem.* **2007**, *55*, 2376−2383.
(14) Su, J. L.; Lin, M. T.; Hong, C. C.; Chang, C. C.; Shiah, S. G.; Wu, C. W.; Chen, S. T.; Chau, Y. P.; Kuo, M. L. Resveratrol induces FasL-related apoptosis through Cdc42 activation of ASK1/JNK-dependent signaling pathway in human leukemia HL-60 cells. *Carcinogenesis* **2005**, *26*, 1−10.
(15) Clement, M. V.; Hirpara, J. L.; Chawdhury, S. H.; Pervaiz, S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. *Blood* **1998**, *92*, 996−1002.
(16) Dorrie, J.; Gerauer, H.; Wachter, Y.; Zunino, S. J. Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells. *Cancer Res.* **2001**, *61*, 4731−4739.
(17) Scarlatti, F.; Maffei, R.; Beau, I.; Codogno, P.; Ghidoni, R. Role of non-canonical Beclin 1-independent autophagy in cell death induced by resveratrol in human breast cancer cells. *Cell Death Differ.* **2008**, *15*, 1318−1329.
(18) Degterev, A.; Yuan, J. Expansion and evolution of cell death programs. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 378−390.
(19) Cregan, S. P.; Dawson, V. L.; Slack, R. S. Role of AIF in caspase-dependent and caspase-independent cell death. *Oncogene* **2004**, *23*, 2785−2796.
(20) Lorenzo, H. K.; Susin, S. A. Therapeutic potential of AIF-mediated caspase-independent programmed cell death. *Drug Resist. Updates* **2007**, *10*, 235−255.
(21) Huang, C. P.; Fang, W. H.; Lin, L. I.; Chiou, R. Y.; Kan, L. S.; Chi, N. H.; Chen, Y. R.; Lin, T. Y.; Lin, S. B. Anticancer activity of botanical alkyl hydroquinones attributed to topoisomerase II poisoning. *Toxicol. Appl. Pharmacol.* **2008**, *227*, 331−338.
(22) Chang, U. M.; Li, C. H.; Lin, L. I.; Huang, C. P.; Lin, S. B. Ganoderiol F, a ganoderma triterpene, induces senescence in hepatoma HepG2 cells. *Life Sci.* **2006**, *79*, 1129−1139.
(23) Salvioli, S.; Ardizzoni, A.; Franceschi, C.; Cossarizza, A. JC-1, but not DiOC6(3) or rhodamine 123, is a reliable fluorescent probe to assess delta psi changes in intact cells: implications for studies on mitochondrial functionality during apoptosis. *FEBS Lett.* **1997**, *411*, 77−82.
(24) van Der Kaaden, J. E.; Hemscheidt, T. K.; Mooberry, S. L. Mappain, a new cytotoxic prenylated stilbene from *Macaranga mappa*. *J. Nat. Prod.* **2001**, *64*, 103−105.
(25) Yoder, B. J.; Cao, S.; Norris, A.; Miller, J. S.; Ratovoson, F.; Razafitsalama, J.; Andriantsiferana, R.; Rasamison, V. E.; Kingston, D. G. Antiproliferative prenylated stilbenes and flavonoids from *Macaranga alnifolia* from the Madagascar rainforest. *J. Nat. Prod.* **2007**, *70*, 342−346.
(26) Su, B. N.; Cuendet, M.; Hawthorne, M. E.; Kardono, L. B.; Riswan, S.; Fong, H. H.; Mehta, R. G.; Pezzuto, J. M.; Kinghorn, A. D. Constituents of the bark and twigs of *Artocarpus dadah* with cyclooxygenase inhibitory activity. *J. Nat. Prod.* **2002**, *65*, 163−169.
(27) Dhingra, V.; Rao, K. V.; Narasu, M. L. Current status of artemisinin and its derivatives as antimalarial drugs. *Life Sci.* **2000**, *66*, 279−300.
(28) Johnstone, R. W.; Ruefli, A. A.; Lowe, S. W. Apoptosis: a link between cancer genetics and chemotherapy. *Cell* **2002**, *108*, 153−164.
(29) Kang, Y. H.; Yi, M. J.; Kim, M. J.; et al. Caspase-independent cell death by arsenic trioxide in human cervical cancer cells: reactive oxygen species-mediated poly(ADP-ribose) polymerase-1 activation signals apoptosis-inducing factor release from mitochondria. *Cancer Res.* **2004**, *64*, 8960−8967.
(30) Sobolev, V. S.; Scott A. Neff, S. A.; Gloer, J. B. New stilbenoids from peanut (*Arachis hypogaea*) seeds challenged by an *Aspergillus caelatus* strain. *J. Agric. Food Chem.* **2009**, *57*, 62−68.

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