JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Selective Growth Inhibition of Human Leukemia and Human Lymphoblastoid Cells by Resveratrol via Cell Cycle Arrest and Apoptosis Induction

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There is great interest in the potential chemopreventive activity of resveratrol against human cancers. However, there are conflicting results on its growth inhibitory effect on normal cells. This project examined the differential effect of resveratrol at physiologically relevant concentrations on nonmalignant (WIL2-NS) and malignant (HL-60) cell lines and compared the underlying mechanisms via cell cycle modulation, apoptosis induction, and genotoxicity potential. Twenty-four hours of exposure to resveratrol was toxic to WIL2-NS and HL-60 cells in a dose-dependent manner. WIL2-NS cells regrew 5 times more than HL-60 cells by 120 h after the removal of 100 μM resveratrol ($p < 0.05$). Furthermore, significant alterations in cell cycle kinetics were induced by resveratrol in HL-60 cells, but were to a lesser extent for WIL2-NS cells. The proportion of apoptosis was also 3 times higher in HL-60 cells as compared to WIL2-NS cells for 100 μM resveratrol ($p < 0.05$). In conclusion, resveratrol preferentially inhibited the growth of HL-60 cells via cell cycle modulation and apoptosis induction and subsequently directed the cells to irreversible cell death, whereas the effect on WIL2-NS cells was largely reversible.

KEYWORDS: Resveratrol; chemopreventive; cell cycle; apoptosis; reversible

INTRODUCTION

Resveratrol, a natural phytochemical, is present in more than 72 plant species, including a wide variety of fruits and vegetables such as grapes, berries, peanut, and various herbs. It has been used extensively in Oriental traditional medicine. The physiological function of resveratrol is to serve as phytoalexin, a defensive agent protecting plants against adverse conditions such as pathogenic attack, mechanical injury, and environmental stressors (*1, 2*).

Jang et al. (*3*) found that in a murine model, resveratrol inhibited diverse cellular events against tumor formation and development induced by the aryl hydrocarbon 7,12-dimethylbenzanthracene (DMBA). Since then, it has been studied extensively in a variety of tumor bioassay models, including breast, prostate, leukemic, melanoma, lung, liver, and colonic tumor cells [reviewed in refs (*1, 2*). The profound cancer chemopreventive potential is associated with its antioxidant (anti-initiation activity), anti-inflammatory (antipromotion activity), growth inhibitory and apoptosis-inducing properties (antiprogression activity), which are relevant to the three general stages of carcinogenesis (*3, 4*).

Effective and applicable chemopreventive agents should have minimal to no toxic effects on nonmalignant and healthy cells. In some reports, resveratrol exhibits its cytotoxicity selectively toward malignant cells with minimal cytotoxicity toward normal blood cells (*5, 6*). However, other studies found that resveratrol, depending upon its concentration, might affect the proliferation of nonmalignant cells. This included keratinocytes (*7*), smooth muscle cells (*8–10*) and endothelial cells (*11*). Obviously these data contradict earlier reports and these growth inhibitory effects might be due to cell-line specificity (*1*). Whereas the growth inhibitory effects are potentially beneficial for chemoprevention, this adverse effect on nonmalignant cells is of great concern. In light of these conflicting data, there is a need for direct comparison of malignant and nonmalignant cells. Moreover, the mechanisms underlying the growth inhibitory effect of resveratrol are not yet clearly defined in nonmalignant cell lines.

In the present study, we tested the roles of resveratrol in vitro on a malignant, promyelocytic leukemia (HL-60) cell line and a transformed nonmalignant, B-cell lymphoblastoid (WIL2-NS) cell line to examine whether resveratrol has a differential effect on the growth inhibition of these two cell lines. The effect of resveratrol on apoptosis induction, genotoxicity and cell cycle modulation was also examined. The hypothesis tested is that resveratrol may exert a selective cytotoxic effect against cancerous cells and have limited to no effect on the growth of nonmalignant cells. It is important to demonstrate whether

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10.1021/jf801014p CCC: $40.75
Published on Web 07/26/2008

resveratrol has significant cytotoxic effects on malignant cells, including what the underlying mechanisms are, and whether it has any effect on nonmalignant cells to justify further clinical trials (*6*).

MATERIALS AND METHODS

Chemicals and Reagents. The chemicals and reagents of analytical grade were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), unless otherwise specified. *trans*-Resveratrol (*trans*-3,5,4′-trihydroxystilbene) and cytochalasin-B (cyt-B) stock solution were prepared in 100% dimethyl sulfoxide (DMSO). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was dissolved in phosphate-buffered saline (PBS). Propidium Iodide (PI)/RNase/Triton-X solution was prepared by adding 100 μL of 1 mg/mL PI and 100 μL of 10 mg/mL RNase into 5 mL of PBS containing 0.1% Triton X-100. The Annexin V-FITC Apoptosis Detection Kit was purchased from BD Biosciences (San Jose, CA). DiffQuick fixative, stain 1, and stain 2 were obtained from Laboratory Aids (Sydney, Australia).

Cell Lines and Culture Conditions. Human promyelocytic leukemia cell line (HL-60) and the transformed, nontumorigenic human B-cell lymphoblastoid cell line (WIL2-NS) were purchased from the American Type Culture Collection (ATCC; Manassas, VA). Both cell lines were maintained in RPMI 1640 (Trace Bioscience, Melbourne, Australia) supplemented with 2 mM L-glutamine, 50 IU/mL penicillin, and 50 μg/mL streptomycin (Thermo Trace, Melbourne, Australia). Medium for WIL2-NS cells was supplemented with 5% heat-inactivated fetal bovine serum (FBS; Trace Biosciences, Australia) and 0.3% β-mercaptoethanol (v/v), whereas 10% heat-inactivated FBS was supplemented in the medium for HL-60 cells. All cell cultures were seeded at 2×10^5 cells/mL in tissue culture flasks and incubated at 37 °C in a humidified 5% CO_2/95% air atmosphere. Both cell lines were subcultured every 2 to 3 days at the same time point for all experiments to ensure the consistency. The cell culture was used for experiments once it had reached 1×10^6 to 2×10^6 cells/mL with 90% viability.

Cell Treatment. Cells were seeded at 1×10^6 cells/mL in a total of 5 mL in 25 cm^2 tissue culture flasks and were treated with resveratrol, ranging from 10 to 500 μM or 1% DMSO as the solvent control (v/v) for 24 h. The treatment medium was then aspirated after centrifugation (Centrifuge 5804, Hamburg, Germany) at 1200 rpm for 5 min and the cells were washed twice with fresh culture media. Each experiment was repeated on at least three independent occasions.

Cytotoxic Studies (MTT assay). The MTT assay was conducted as described by Young et al. (*12*). After treatments, 1×10^4 cells/100 μL were plated in 96-well microplates with 0.5 mg/mL MTT solution for 18 h. Then, 20% SDS in 0.02 M HCl was added for 1.5 h at room temperature (RT) in the dark. The optical density (OD) was measured at 570 nm with a multiwell plate spectrophotometer reader (Biotek Instruments, Moorebank, NSW). A standard curve was conducted for each experiment and used to convert treatment ODs to viable cells/well. Cell survivals were expressed as percentage of viability compared to the control (*12*).

Cytostatic Studies (Population Growth Assay). Three concentrations (10, 50, and 100 μM) at which cell lines were significantly killed by resveratrol in the MTT assay (**Figure 1**, $p < 0.05$), were selected to test for the reversibility of cell growth inhibitory effects. Briefly, suspension cells were mixed 1:1 with 0.2% Trypan Blue and counted using a hemocytometer to estimate viable cells/mL at the end of the treatment (the Trypan Blue Exclusion test). The cell concentration was adjusted to 1×10^6 cells/5 mL and cultures were grown for 120 h in 25 cm^2 flasks for the solvent control and the treatments. Cell concentration was determined at 72 and 120 h using the same method. Five mL fresh medium was added at 72 h to the cell cultures. Cell concentration was converted to total population size by multiplying viable cells/mL by medium volume.

Cell Cycle Distribution Analysis. To assess whether resveratrol-induced growth inhibition of the cells is mediated via alterations in cell cycles, the effect of resveratrol on cell cycle distribution was evaluated. This method was conducted as described by Nicoletti et al. (*13*) with minor modifications. After treatment, 100 μL of 1×10^6 treated or solvent cells were fixed by addition of 3 mL of cold 70% ethanol and the samples were then stored at −20 °C overnight. Prior to staining, cells were washed with PBS/1% Sodium Azide (AZ) and then resuspended in 1 mL of PI/RNase/Triton X-100 solution. After incubation for 30 min at RT in the dark, the fluorescence of stained cells was analyzed by Fluorescence Activated Cell Sorting (FACScan) flow cytometry (Becton & Dickson, San Jose, CA) and relative gated cells in each cell cycle phase were determined. Data acquisition and analysis were performed using CellQuest software (Becton & Dickson) for 20000 cellular events per sample.

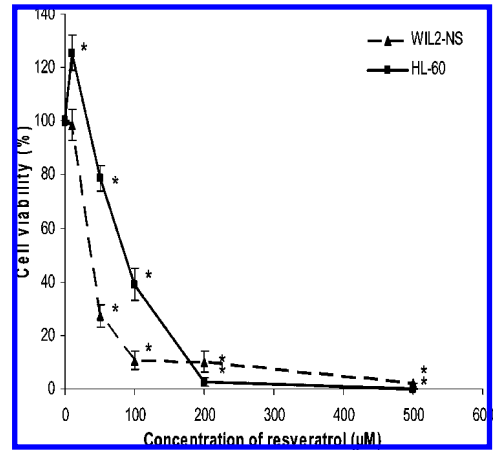

Figure 1. Relative survival after 24 h of treatment with resveratrol on WIL2-NS and HL-60 cells. Cell viability was measured by the MTT assay. Cell survival is expressed as percentage of viable cells/well compared to the solvent control (1% DMSO) as 100%. Data are the mean ± standard error of at least four independent experiments ($n \geq 4$). Treatments significantly different from solvent control at $p < 0.05$ are presented as ∗.

Apoptosis Induction Assay. The Annexin V-FITC Apoptosis Detection Kit was used as described by the manufacturer's protocol to determine the percentage of cells undergoing apoptosis. It detects phosphatidylserine externalisation of the cell membrane in early stages of apoptosis (*14*). Briefly, cells after treatment were washed in cold PBS/AZ and then resuspended in 1× binding buffer. Then, 1×10^5 cells were double stained with 5 μL of Annexin V-FITC and 5 μL of PI for 15 min at RT in the dark. To this mixture was added 400 μL of 1× binding buffer before analysis using FACScan flow cytometry within one hour. Twenty thousand cellular events in each sample were analyzed using CellQuest software.

Genotoxicity Assay. The genotoxic potential of resveratrol to induce chromosome damage in cells was examined as described by Fenech et al. (*15*) with minor modifications. Briefly, cyt-B at 4.5 μg/mL was added to cultures at 0.5×10^6 cells/mL after treatment. Cells were then incubated for 26 h at 37 °C. The cells were harvested by transferring directly onto a slide using a cytospin (Shando Scientific, Astmoor, Runcorn, U.K.) at 600 rpm for 5 min. The slides were air-dried, fixed and stained using DiffQuick fixative for 10 min, then 10 times 1 s of stain 1 (red) followed by stain 2 (blue) for 10 times 1 s. The slides were scored at 250× magnification by light microscopy for micronucleated binucleated cells (MNed BNC). Frequency of MNed BNC in 1000 BNC was determined according to criteria described by Fenech et al. (*15*).

Statistical Analysis. All experiments were performed on at least three independent occasions. Data were analyzed by one-way analysis of variance (ANOVA) for MTT and cell cycle analysis followed by Tukey's multiple-range tests for significant differences. Statistical significance between two cell lines in MTT was determined by Independent *t* test. Two-way analysis of variance (ANOVA) was performed for population growth and apoptosis induction assays followed by pairwise comparison for the simple main effect for significant differences between the control and the treatment as well as the two cells lines. Treatment values with $p < 0.05$ were considered statistically different.

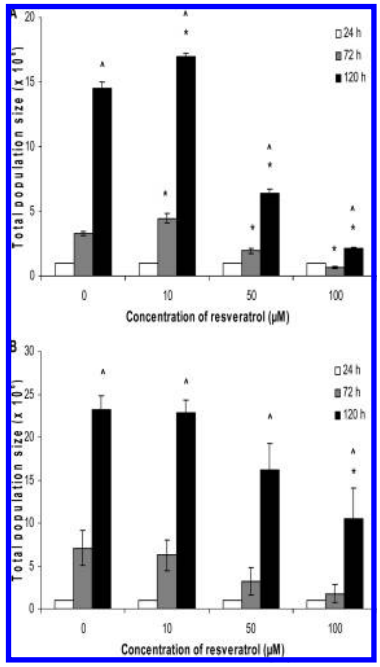

Figure 2. Growth of cell cultures of (**A**) HL-60 and (**B**) WIL2-NS cells after the removal of resveratrol following a 24 h exposure. Cells were sampled after 72 and 120 h and counted with the Trypan Blue Exclusion test. The values of total population size are expressed as total viable cells in 5 mL of medium. Data are the means ± standard error of three independent experiments ($n = 3$). Treatments significantly different from solvent control at the same time point at $p < 0.05$ are presented as ∗. Treatments significantly different between the two cell lines at the same concentration at $p < 0.05$ are presented as ^.

RESULTS

Cytotoxicity Effect of Resveratrol. As seen in **Figure 1**, resveratrol showed a dose-dependent inhibitory effect on the cell proliferation of WIL2-NS and HL-60 cells assessed by the MTT assay. After 24 h of exposure, resveratrol significantly suppressed the cell growth of WIL2-NS and HL-60 at concentrations greater than 50 μM ($p < 0.05$). Treatment with 50 μM resveratrol inhibited the cell growth of WIL2-NS by 73% ($p < 0.05$) and the cell growth of HL-60 by 21% ($p < 0.05$). The median inhibitory concentration (IC_{50}) values were 30 μM and 80 μM for WIL2-NS and HL-60 cells, respectively. The response of HL-60 cells to treatment with resveratrol was biphasic. The low concentration of 10 μM resveratrol increased cell numbers by 25% as compared to the solvent control ($p < 0.05$). At 200 μM, HL-60 cells appeared to be more sensitive to the cytotoxicity effect of resveratrol than WIL2-NS cells, but the difference is not statistically significant.

Cytostatic Effect of Resveratrol. Growth inhibitory effect of resveratrol was reversible depending on the doses of resveratrol applied. After treatment with resveratrol for 120 h, population size of WIL2-NS cells ranged from 23×10^6 to 10×10^6 cells (statistically different from the solvent control at 100 μM for 120 h) as shown in **Figure 2**. When HL-60 cells were treated with resveratrol, cell growth did not recover even after 120 h, with no reversible growth observed ($p < 0.05$ for 50 and 100 μM resveratrol). The greatest growth inhibition effect was observed at 100 μM resveratrol in HL-60 cells where the cell population size was reduced from 14×10^6 to 2×10^6cells at 120 h. WIL2-NS cells regrew 5 times more than HL-60 cells by 120 h after the removal of 100 μM resveratrol ($p < 0.05$).

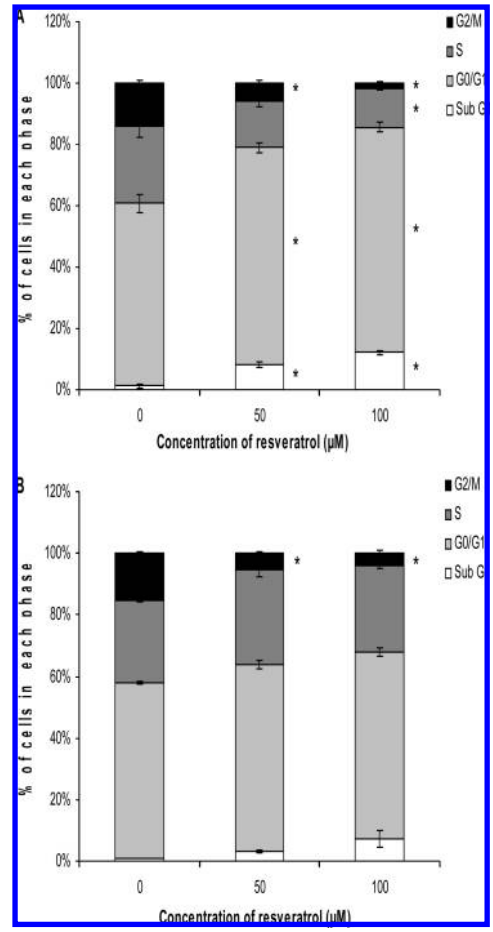

Figure 3. Cell cycle phase distribution of (**A**) HL-60 and (**B**) WIL2-NS cells after a 24 h exposure in the presence or absence of 50 and 100 μM resveratrol. Cells were stained with PI and analyzed by flow cytometry. The values are given as the percentage of cells gated in each phase. Data are the mean ± standard error of three independent experiments ($n = 3$). Treatments significantly different from solvent control at $p < 0.05$ are presented as ∗.

Cell Cycle Arrest by Resveratrol. Cell cycle distribution analysis revealed the accumulation of HL-60 cells in G0/G1 phase after treatment with resveratrol as compared with the solvent control (**Figure 3**). The histogram of DNA content in HL-60 cells treated with 100 μM resveratrol showed a significant increase in the G0/G1 peak from 61 to 78%, whereas the population of cells in S and G2/M phase reduced significantly from 26 to 13% and from 15 to 2% as compared to the solvent control, respectively ($p < 0.05$). WIL2-NS cells treated with resveratrol showed only a small nonsignificant accumulation of cells in the G0/G1 phase. There was a significant reduction of G2/M cells in WIL2-NS cells, which decreased from 15.7 to 4% for 100 μM as compared to the solvent control ($p < 0.05$). Sub-G1 peaks indicative of apoptosis were observed in the HL-60 cells ($p < 0.05$), but not in the WIL2-NS cells. The sub-G1 population of HL-60 cells increased significantly from 1% at 0 μM to 8% at 50 μM and 12% at 100 μM resveratrol as compared to the solvent control ($p < 0.05$).

Induction of Apoptosis by Resveratrol. The apoptosis induced by resveratrol on HL-60 and WIL2-NS cell lines was quantified by flow cytometry as shown in **Figure 4**. Annexin-V positive cells represented 0.8% of the whole cell population of HL-60 cells at 0 μM, which increased to 4.2% at 50 μM and 19.8% at 100 μM resveratrol indicating significant apoptosis induction for both doses as compared to the solvent control (p

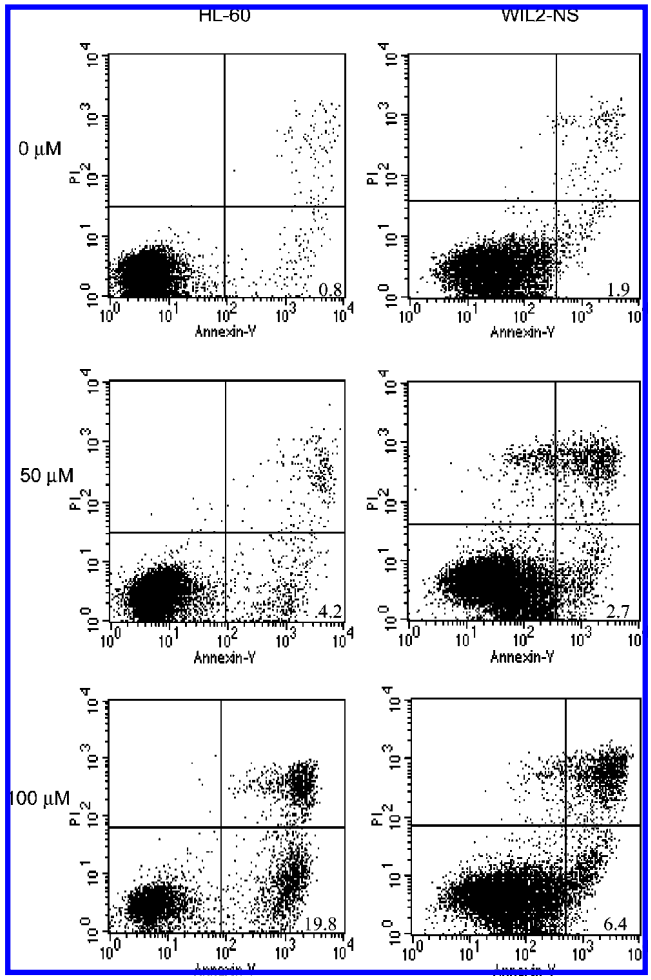

Figure 4. Flow cytometric analysis of viable, apoptotic, and necrotic cells of HL-60 and WIL2-NS cells after 24 h of treatment with resveratrol. The lower left corner of each scatter plot indicates the viable cells (negative for both Annexin V-FITC and PI). The lower right corner represents the early apoptotic cells (positive for Annexin V-FITC, but not PI). The upper right corner shows the necrotic cells (double positive for both staining). The numbers in the figure represent the percentage of cells in each population. Two $\times$ 10^4 events were collected and analyzed by flow cytometry.

< 0.05). For WIL2-NS cells treated with 50 and 100 μM resveratrol, the apoptosis population increased from 1.9% of the control to 2.7% and 6.4% at 50 and 100 μM resveratrol, respectively (not statistically significant). After subtracting the basal level of apoptosis in the untreated population according to the suggested protocol by the manufacturer, the percentages of apoptotic cells induced by 100 μM resveratrol were 4.5% for WIL2-NS and 18.9% for HL-60 cells. DNA fragmentation as revealed in the cell cycle studies confirmed that resveratrol induced apoptosis in both cell lines. HL-60 cells, when compared to WIL2-NS cells, were found to be more sensitive to the induction of apoptosis by resveratrol treatment at 100 μM with a significantly 3 times higher proportion of the cell population undergoing apoptosis after 24 h of treatment ($p <$ 0.05).

Genotoxicity Potential of Resveratrol. The CBMN experiments performed after the 24 h exposure of resveratrol (50 and 100 μM) are shown in **Table 1**. The results revealed no significant increase of MNed BNC in either cell line. The highest number of MNed BNC observed in HL-60 cells was 2 MNed BNC/1000 BNC at 50 μM resveratrol and for WIL2-NS cells was 4 MNed BNC/ 1000 BNC at 10 μM resveratrol. In order to confirm the sensitivity of all experiments, 80 μM of H_2O_2 was used in parallel as a positive control. It induced a significant number of MNed BNC/1000 BNC ($p < 0.05$).

Table 1. Numbers of Micronucleated Binucleated Cells (MNed BNC) Counted in HL-60 and WIL2-NS Cells after 24 h of Exposure to 10−100 μM Resveratrol and Hydrogen Peroxide (H_2O_2) (250$\times$ Magnification)

		MNed BNC/1000 BNC[a]	
treatment	concn (μM)	HL-60	WIL2-NS
resveratrol	0	0.33 ± 0.33	2.67 ± 0.67
	10	0.00 ± 0.00	4.00 ± 1.53
	50	2.00 ± 1.15	2.67 ± 0.67
	100	1.00 ± 0.88	3.67 ± 1.33
H_2O_2	80	22.67 ± 1.20[b]	69.67 ± 1.76[b]

[a] Values are the mean ± standard error of MNed per 1000 BNC in three independent experiments ($n = 3$). [b] Treatments significantly different from the solvent control at $p < 0.05$.

DISCUSSION

Resveratrol has been regarded as a remarkable chemopreventive drug candidate and has been reviewed extensively, due to its diverse biological activity against various cancerous cells in numerous in vitro and in vivo studies [reviewed in refs (*1, 2*).]. However, several studies indicated that the resveratrol might have an adverse effect on nonmalignant cells (reviewed in ref *1*). This growth inhibition effect on nonmalignant cells has the potential to produce undesirable side effects, which limit the pharmacological application of resveratrol to be used safely in humans. On the basis of these previous studies, we tested if the differential growth inhibitory effect of resveratrol exists between malignant and nonmalignant cells, and examined the possible mechanisms of action. We hypothesized that resveratrol may possess a selective cytotoxic effect toward human cancer cells as compared to nonmalignant origin human cells.

The current study found that resveratrol inhibited the proliferation of both nonmalignant and malignant cell lines consistent with some previous studies (*9, 10, 16*). The effect of resveratrol was considered to be cell type- and dose-dependent, as seen by the different susceptibility of the two cell lines tested in this study. Doses of resveratrol (micromolar range) used in the experiments are comparable to the concentrations of resveratrol accumulated in liver and kidney following oral administration to mice (*17, 18*). WIL2-NS, nonmalignant origin cells, displayed a higher sensitivity to resveratrol in the MTT assay at concentrations <200 μM, as shown by lower IC_{50} values, when compared to the HL-60 cell line (**Figure 1**). This might be due to the higher proliferation rate of WIL2-NS cells as compared to the HL-60 cell line (data not shown). Therefore, these cells may absorb and metabolize resveratrol more rapidly, and manifest a stronger cytotoxic effect. The stimulation of proliferation at low concentration (10 μM) against HL-60 cells indicated the biphasic effects of resveratrol in a dose-dependent manner. It has been previously found that concentrations <25 μM of resveratrol have a stimulatory effect on growth of ER^+ breast cancer cells (*19*). However, this effect was cell type-dependent and not observed in WIL2-NS cells. At concentrations greater than 200 μM, the cytotoxicity effect of resveratrol is not statistically significant different between WIL2-NS and HL-60 cells.

The long-term proliferation capacity tests (cytostatic) for up to 120 h demonstrated the ability of the WIL2-NS cell line to recover from the cytotoxic effect of resveratrol after the removal of resveratrol (**Figure 2**). This might be due to the ability of

WIL2-NS cells to repair DNA damage to avoid cells undergoing the process of cell death. This phenomenon needs to be followed up with additional nonmalignant cell types to determine if this effect is cell-type dependent. The irreversible effect of resveratrol in HL-60 revealed the growth inhibition of cells might be by mechanisms different from that for nonmalignant cells. This possibility was investigated by additional assays.

As revealed by flow cytometry analysis, suppression of cell proliferation with resveratrol was accompanied by the significant accumulation of cells in the G1/S phase boundary in HL-60 cells (**Figure 3**). Resveratrol at 50 μM and 100 μM blocked G1-S transition, resulting in G0/G1 phase cell cycle arrest, thereby delaying the progression of cells through S and G2/M phase in HL-60 cells. This was shown by a significant decrease in the proportion of cells in S phase, and the almost absence of G2/M phase cell population. In contrast, resveratrol showed partial blockage of WIL2-NS cells in G0/G1 phase, in parallel with the significant reduction of cell population in G2/M phase at 50 and 100 μM (not significant for G0/G1 phase arrest). A temporary effect of cell cycle arrest at particular phases allows time for repair of some cellular damages, primarily to DNA, which then enables cell survival (*20*). The reversible proliferation effect as seen in WIL2-NS cells might be associated with the restoration of cell cycle progression after the removal of resveratrol as observed by Haider et al. (*8*) in vascular smooth muscle cells.

There are many possible different explanations for the cell cycle blockage ability of resveratrol. The effect is likely related to the direct involvement of resveratrol in DNA replication via inhibition of ribonucleotide reductase (*10*) and DNA polymerase activities (*21*), which are essential for DNA synthesis. Many other studies also correlated resveratrol effects to its regulation of key proteins in the cell cycle via the decrease in NF-κB activity (*22*) or the increase in p53 and $p21^{WAF1/CIP1}$ expression (*9, 23*). Results with regard to cell cycle arrest caused by resveratrol are highly variable in different cell types and even in the same cells, as shown by either G0/G1 phase arrest in A431 epidermoid carcinoma cells (*23*) and CEM-C7H2 acute leukemia cells (*24*), S phase arrest in MCF7 breast carcinoma cells (*25*), and LNCap prostate cancer cells (*26*) or G2/M phase arrest in early myeloid leukemic cells (K562, KCL22) (*16*). The current results suggested that cell cycle arrest might be a major determinant in the growth inhibitory effect of resveratrol.

The DNA fragmentation fractions (subdiploid DNA contents in flow cytometry analysis) as indicated in the sub-G1 population are an indicator of DNA fragmentation associated with apoptosis (*13*). The gradual increase in the proportion of sub-G1 phase was concomitant with the increase in the frequency of apoptosis cells in HL-60 but not in WIL2-NS cells ($p < 0.05$).

Perturbation of cell cycle is a prerequisite for the induction of apoptosis (*20*). The results from the current apoptosis study were consistent with previous finding (*6*), which have indicated the ability of resveratrol to induce apoptosis at higher concentrations (100 μM). Resveratrol at 100 μM showed 3 times higher apoptotic cell death (19.8%) in HL-60 than WIL2-NS cells (6.4%, $p < 0.05$). In contrast to the response of HL-60 cells, the apoptosis induction in WIL2-NS cells by resveratrol was at a lower level and not statistically significant as compared to the solvent control (**Figure 4**).

The ability to induce DNA damage to the cancerous cells at chromosomal levels (genotoxicity) is one of the promising mechanisms that contribute to the chemopreventive properties (*27*). Therefore, the genotoxicity potential of resveratrol was examined. Results in the current study from the CBMN assay indicated that resveratrol does not induce chromosome damage in nonmalignant and malignant cell lines (**Table 1**), and were consistent with the findings of Salvia et al. (*28*). Furthermore, resveratrol was previously found to protect the genome by the ability to repair DNA damage rather than induce DNA damage to cells (*29*). There was no significant increase in MNed BNC induced by any resveratrol treatment in the current study. Genetic damages manifested by micronuclei (MN) are chromosomal loss, chromosome breakage lacking centromeres and whole chromosomes that are unable to reach the spindle poles during mitosis (*15*). In contrast, previous findings have demonstrated that resveratrol binds to DNA in the presence of copper ions (Cu^{2+}), and induces a copper-peroxide complex leading to Cu^{2+}-dependent DNA damage and DNA strand breakage in cells (*30*). The medium used in the current experiments (RPMI-1640) did not contain any Cu^{2+}; therefore, it is reasonable to suggest that no binding would occur between resveratrol and DNA under the conditions used. This hypothesis can be tested using media that contains copper ions (Cu^{2+}).

In conclusion, this study showed that resveratrol inhibited the growth of malignant cells and nonmalignant cells. However, cell cycle arrest and apoptosis induction were significantly higher ($p < 0.05$) in malignant cells and subsequently caused irreversible cell death in the HL-60 cells. In contrast, the nonmalignant cells WIL2-NS, with lower apoptosis induction and almost no cell cycle arrest, recovered from the inhibitory effect after the removal of resveratrol. These results indicated the possibility of minimal cytotoxic effect of resveratrol on nonmalignant cells and therefore supported the potential use of resveratrol as a chemoprevention agent. In relation to this, the difference in the mechanism of action of resveratrol toward nonmalignant and malignant cells is very interesting and deserves further investigation to confirm if this is a general phenomenon.

ACKNOWLEDGMENT

We thank Kylie Lange for her kind help with the statistical analysis and Sheree Bailey from the Department of Immunology, Allergy and Arthritis, Flinders Medical Centre, for helpful assistance with flow cytometry.

LITERATURE CITED

(1) Aggarwal, B. B.; Bhardwaj, A.; Aggarwal, R. S.; Seeram, N. P.; Shishodia, S.; Takada, Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res.* **2004**, *24*, 3–60.

(2) De la Lastra, C. A.; Villegas, I. Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. *Mol. Nutr. Food Res.* **2005**, *49*, 405–430.

(3) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W.; Fong, H. H.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **1999**, *275*, 218–220.

(4) Surh, Y. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. *Mutat. Res.* **1999**, *428*, 305–327.

(5) Dörrie, 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.

(6) Clément, M. V.; Hirpara, J. L.; Chawdhury, S. H.; Pervaiz, S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signalling-dependent apoptosis in human tumor cells. *Blood* **1998**, *92*, 996–1002.

(7) Holian, O.; Walter, R. J. Resveratrol inhibits the proliferation of normal human keratinocytes in vitro. *J. Cell. Biochem. Suppl.* **2001**, *36*, 55–62.
(8) Haider, U. G.; Sorescu, D.; Griendling, K. K.; Vollmar, A. M.; Dirsch, V. M. Resveratrol increases serine15-phosphorylated but transcriptionally impaired p53 and induces a reversible DNA replication block in serum-activated vascular smooth muscle cells. *Mol. Pharmacol.* **2003**, *63*, 925–932.
(9) Mnjoyan, Z. H.; Fujise, K. Profound negative regulatory effects by resveratrol on vascular smooth muscle cells: a role of p53-p21(WAF1/CIP1) pathway. *Biochem. Biophys. Res. Commun.* **2003**, *311*, 546–552.
(10) Zou, J.; Huang, Y.; Chen, Q.; Wang, N.; Cao, K.; Hsieh, T. C.; Wu, J. M. Suppression of mitogenesis and regulation of cell cycle traverse by resveratrol in cultured smooth muscle cells. *Int. J. Oncol.* **1999**, *15*, 647–651.
(11) Szende, B.; Tyihák, E.; Király-Véghely, Z. Dose-dependent effect of resveratrol on proliferation and apoptosis in endothelial and tumor cell cultures. *Exp. Mol. Med.* **2000**, *32*, 88–92.
(12) Young, F. M.; Phungtamdet, W.; Sanderson, B. J. Modification of MTT assay conditions to examine the cytotoxic effects of amitraz on the human lymphoblastoid cell line, WIL2NS. *Toxicol. In Vitro* **2005**, *19*, 1051–1059.
(13) Nicoletti, I.; Migliorati, G.; Pagliacci, M. C.; Grignani, F.; Riccardi, C. A rapid and simple method for measuring thymocyte apoptosis by propidium iodide staining and flow cytometry. *J. Immunol. Methods* **1991**, *139*, 271–279.
(14) Vermes, I.; Haanen, C.; Steffens-Nakken, H.; Reutelingsperger, C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *J. Immunol. Methods* **1995**, *184*, 39–51.
(15) Fenech, M.; Chang, W. P.; Kirsch-Volders, M.; Holland, N.; Bonassi, S.; Zeiger, E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat. Res.* **2003**, *534*, 65–75.
(16) Ferry-Dumazet, H.; Garnier, O.; Mamani-Matsuda, M.; Vercauteren, J.; Belloc, F.; Billiard, C.; Dupouy, M.; Thiolat, D.; Kolb, J. P.; Marit, G.; Reiffers, J.; Mossalayi, M. D. Resveratrol inhibits the growth and induces the apoptosis of both normal and leukemic hematopoietic cells. *Carcinogenesis* **2002**, *23*, 1327–1333.
(17) Signorelli, P.; Ghidoni, R. Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. *J. Nutr. Biochem.* **2005**, *16*, 449–466.
(18) Vitrac, X.; Desmoulière, A.; Brouillaud, B.; Krisa, S.; Deffieux, G.; Barthe, N.; Rosenbaum, J.; Mérillon, J. M. Distribution of [^{14}C]-*trans*-resveratrol, a cancer chemopreventive polyphenol, in mouse tissues after oral administration. *Life Sci.* **2003**, *72*, 2219–2233.
(19) Nagakawa, H.; Kiyozuka, Y.; Uemura, Y.; Senzaki, H.; Shikata, N.; Hioki, K.; Tsubura, A. Resveratrol inhibits human breast cancer cell growth and may mitigate the effect of linoleic acid, a potent breast cancer cell stimulator. *J. Cancer Res. Clin. Oncol.* **2001**, *127*, 258–264.
(20) Hickman, J. A. Apoptosis induced by anticancer drugs. *Cancer Metastasis Rev.* 1992*11*, 121–139.
(21) Locatelli, G. A.; Savio, M.; Forti, L.; Shevelev, I.; Ramadan, K.; Stivala, L. A.; Vannini, V.; Hübscher, U.; Spadari, S.; Maga, G. Inhibition of mammalian DNA polymerases by resveratol: mechanism and structural determinants. *Biochem. J.* **2005**, *389*, 259–268.
(22) Holmes-McNary, M.; Baldwin, A. S., Jr. Chemopreventive properties of *trans*-resveratrol are associated with inhibition of activation of the IkappaB kinase. *Cancer Res.* **2000**, *60*, 3477–3483.
(23) Ahmad, N.; Adhami, V. M.; Afaq, F.; Feyes, D. K.; Mukhtar, H. Resveratrol causes WAF-1/p21-mediated G(1)-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. *Clin. Cancer Res.* **2001**, *7*, 1466–1473.
(24) Bernhard, D.; Tinhofer, I.; Tonko, M.; Hübl, H.; Ausserlechner, M. J.; Greil, R.; Kofler, R.; Csordas, A. Resveratrol causes arrest in the S-phase prior to Fas-independent apoptosis in CEM-C7H2 acute leukemia cells. *Cell Death Differ.* **2000**, *7*, 834–842.
(25) Pozo-Guisado, E.; Alvarez-Barrientos, A.; Mulero-Navarro, S.; Santiago-Josefat, B.; Fernandez-Salguero, P. M. The antiproliferative activity of resveratrol results in apoptosis in MCF-7 but not in MDA-MB-231 human breast cancer cells: cell-specific alteration of the cell cycle. *Biochem. Pharmacol.* **2002**, *64*, 1375–1386.
(26) Kuwajerwala, N.; Cifuentes, E.; Gautam, S.; Menon, M.; Barrack, E. R.; Reddy, G. P. Resveratrol induces prostate cancer cell entry into s phase and inhibits DNA synthesis. *Cancer Res.* **2002**, *62*, 2488–2492.
(27) Benner, S. E.; Wargovich, M. J.; Lippman, S. M.; Hong, W. K. Micronuclei: a potential intermediate marker for chemoprevention of aerodigestive tract cancer. *J. Cell. Biochem. Suppl.* **1993**, *17F*, 250–254.
(28) De Salvia, R.; Festa, F.; Ricordy, R.; Perticone, P.; Cozzi, R. Resveratrol affects in a different way primary versus fixed DNA damage induced by H_2O_2 in mammalian cells in vitro. *Toxicol. Lett.* **2002**, *135*, 1–9.
(29) Usha, S.; Johnson, I. M.; Malathi, R. Interaction of resveratrol and genistein with nucleic acids. *J. Biochem. Mol. Biol.* **2005**, *38*, 198–205.
(30) Azmi, A. S.; Bhat, S. H.; Hadi, S. M. Resveratrol-Cu(II) induced DNA breakage in human peripheral lymphocytes: implications for anticancer properties. *FEBS Lett.* **2005**, *579*, 3131–3135.

Received for review April 2, 2008. Revised manuscript received June 4, 2008. Accepted June 13, 2008.

JF801014P