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Resveratrol Triggers Apoptosis Through Regulating Ceramide Metabolizing Genes in Human K562 Chronic Myeloid Leukemia Cells

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Resveratrol, an important phytoalexin in many plants, has been reported to have cytotoxic effects on various types of cancer. Ceramide is a bioactive sphingolipid that regulates many signaling pathways, including cell growth and proliferation, senescence and quiescence, apoptosis, and cell cycle. Ceramides are generated by longevity assurance genes (LASS). Glucosylceramide synthase (GCS) and sphingosine kinase-1 (SK-1) enzymes can convert ceramides to antiapoptotic molecules, glucosylceramide, and sphingosine-1-phosphate, respectively. C8:ceramide, an important cell-permeable analogue of natural ceramides, increases intracellular ceramide levels significantly, while 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) and SK-1 inhibitor increase accumulation of ceramides by inhibiting GCS and SK-1, respectively. Chronic myelogenous leukemia (CML) is a hematological disorder resulting from generation of BCR/ABL oncogene. In this study, we examined the roles of ceramide metabolizing genes in resveratrol-induced apoptosis in K562 CML cells. There were synergistic cytotoxic and apoptotic effects of resveratrol with coadministration of C8:ceramide, PDMP, and SK-1 inhibitor. Interestingly, there were also significant increases in expression levels of LASS genes and decreases in expression levels of GCS and SK-1 in K562 cells in response to resveratrol. Our data, in total, showed for the first time that resveratrol might kill CML cells through increasing intracellular generation and accumulation of apoptotic ceramides.

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INTRODUCTION

Resveratrol (3,5,4′-trans-trihydroxystilbene) is a natural product generated in various plants, especially in the skin and seeds of red grapes, to protect them in stress conditions, such as fungal infections or UV irradiation (1,2). Resveratrol has drawn increasingly more attention recently since it has antiinflammatory, antimetastatic, cardioprotective, chemopreventive, cancer-preventive, apoptosis inducer, and radio-sensitizer potentials (3–5). Resveratrol affects the processes of tumor initiation, promotion, and progression (6). It is very well known that resveratrol can bind directly to DNA and RNA. Therefore, it can protect DNA from genotoxic effects of mutagens. On the other hand, resveratrol can also inhibit DNA topoisomerase II enzyme and induce apoptosis (6). Different mechanisms were explained for resveratrol-induced apoptosis, such as repression of NF-κB and STAT3 pathways and downregulation of cyclin D1, Bcl-2, Bcl-XL, XIAP, and AKT genes (7). We have previously showed the antiproliferative effect of resveratrol on cultured chronic myeloid leukemia (CML) cells (1). CML is a clonal hematological disorder, which raises from reciprocal translocation between the BCR (break point cluster) gene on chromosome 22 and the ABL (Abelson leukemia virus oncogene) gene on chromosome 9, t(9,22)(q34,q11), resulting in the formation of BCR/ABL fusion protein (8). This fusion protein possesses constitutive tyrosine kinase activity (9). Furthermore, BCR/ABL induces several signal transduction pathways that mediate cellular proliferation and cause the disruption of the genetic maintenance. Although there are very effective treatment agents targeting BCR/ABL oncogene, emergence of the resistance is a significant problem for the treatment of CML. However, the roles of bioactive

sphingolipids in resveratrol-induced apoptosis in cultured CML cells have not been examined previously.

Ceramide, the most important bioactive sphingolipid, constitutes a signaling mediator of various biological activities, including cell cycle arrest, cell senescence, cell migration and adhesion, differentiation, and apoptosis in various types of normal and tumor cells (10). It has been shown that intracellular concentrations of ceramides are increased in response to chemotherapy, ionizing radiation and several environmental stress conditions (11). Ceramide levels are regulated by longevity assurance genes (LASS) that synthesize ceramides and by glucosylceramide synthase (GCS) and sphingosine kinase-1 (SK-1) that convert apoptotic ceramides to antiapoptotic glucosylceramide and sphingosine-1-phosphate (S1P), respectively (12). Unlike ceramides, increasing concentrations of glucosylceramide and S1P in the cell promote cell growth and proliferation (12). In addition, it has been reported that elevated levels of GCS and S1P cause multidrug resistance in many cancer cells (12–14). We have previously shown that ceramides have significant roles in the sensitivity and resistance of CML cells to imatinib (10). In addition, there are some other studies showing the effects of resveratrol on ceramide generation. Ersilia Dolfini et al. have shown that resveratrol may prevent the growth of MDA-MB-231 breast cancer cells through inducing de novo ceramide generation (15). In another study, it was also shown that resveratrol triggers autophagy in gastric cancer cells via increasing intracellular ceramide levels (16).

We and some others have reported that targeting ceramide metabolism induces apoptotic signaling pathways and, therefore, promotes apoptosis in various types of cancer, including CML (10,17). In this study, our hypothesis was that ceramide generating and ceramide clearance genes may be involved in resveratrol-induced apoptosis. We also hypothesized that targeting bioactive sphingolipid metabolism by mimetics/inhibitors that trigger ceramide generation and accumulation can increase apoptotic effects of resveratrol on K562 CML cells.

MATERIALS AND METHODS

Cell Line and Chemicals

Human K562 CML cells were obtained from German Collection of Microorganisms and Cell Cultures (Germany). Resveratrol (trans-3,4′, 5-trihydroxystilbene) was obtained from Sigma Aldrich (St. Louis, MO). The stock solution of resveratrol was dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mmol/mL, stored at −20°C, and diluted in cell culture medium. C8:ceramide, 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), and SK-1 inhibitor were obtained from Cayman Chemicals (Ann Arbor, MI) and dissolved in DMSO. Penicillin-streptomycin, RPMI1640, and fetal bovine serum were obtained from Invitrogen (Paisley, UK).

Culture Conditions

The Ph (+) human K562 CML cells were cultured in RPMI1640 growth medium containing 10% fetal bovine serum and 1% penicillin-streptomycin at 37°C in 5% CO_2.

Measurement of Cell Growth by XTT Assay

The IC50 values (drug concentration that inhibits cell growth by 50%) of resveratrol and C8:ceramide, and the IC10 values (drug concentration that inhibits cell growth by 10%) of PDMP and SK-1 inhibitor were determined by XTT cell proliferation assay. In short, 2×10^4 cells/well were seeded into 96-well plates containing 100 μl of the growth medium in the absence or presence of increasing concentrations of resveratrol, C8:ceramide, PDMP, or SK-1 inhibitor and then incubated at 37°C in 5% CO_2. After 72 h incubation period, cells were treated with 50 μl XTT for 4 h. Then, the plates were read under 492-nm wavelengths by ELISA reader (Thermo Electron Corporation Multiskan Spectrum, Vantaa, Finland). Finally, IC50 values of resveratrol and C8:ceramide, and IC10 values of PDMP and SK-1 inhibitor were calculated from the cell proliferation plots. We prefer to combine increasing concentrations of resveratrol with IC10 values of PDMP and SK-1 inhibitor since we have shown previously that IC10 values of these chemicals are enough to inhibit the enzyme activity and provide accumulation of ceramides in the cells (10). On the other hand, since C8:ceramide by itself triggers induction of generation of apoptotic ceramide, it can be accepted like a pro-drug. Therefore, we combined the IC50 value of C8:ceramide with increasing concentrations of resveratrol. Our main purpose in these combinations was to see if we could enhance the sensitivity of K562 cells to resveratrol by increasing intracellular concentrations of ceramides.

In order to determine the possible synergistic effects of resveratrol in combination with C8:ceramide, PDMP, or SK-1 inhibitor, 2×10^4 K562 cells were seeded into each well of a 96-well plate containing 100 μl of the growth medium. Then IC50 value of C8:ceramide or IC10 value of PDMP or SK-1 inhibitor were applied onto the cells, and the plates were incubated at 37°C in 5% CO_2 for 18 h. This time period provides increased generation of ceramides through its induction by C8:ceramide or increased accumulation of ceramides through inhibition of conversion of ceramides to glucosylceramide or sphingosine-1-phosphate by PDMP or SK-1 inhibitor, respectively. Increasing concentrations of resveratrol were then applied into these plates, and they were incubated at 37°C in 5% CO_2. After 72 h incubation, cells were treated with 50 μl of XTT mixture for 4 h and the plates were read under 492 nm wavelengths by ELISA reader (Thermo Electron Corporation Multiskan Spectrum, Vantaa, Finland).

Evaluation of Apoptosis

Changes in caspase-3 enzyme activity of the cells, an important sign of apoptosis, were examined by caspase-3 colorimetric

assay kit (BioVision Research Products, Mountain View, CA). This assay is based on spectrophotometric detection of the chromophore *p*-nitroanilide (*p*NA) after cleavage from the labeled substrate DEVD-*p*NA that can be recognized by caspases. In short, the cells (1×10^6 cells/2 mL/well), induced to undergo apoptosis, were collected by centrifugation at 1,000 rpm for 10 min. The cells were lysed by adding 50 μl of chilled cell lysis buffer and incubated on ice for 10 min before centrifugation at 10,000 *g* for 1 min. Supernatants were transferred to new Eppendorf tubes, and the reaction mixture was prepared in 96-well plates by adding 50 μl of 2× reaction buffer (containing 10 mM DTT), 50 μl of sample, and 5 μl of DEVD-pNA substrate and incubated for 2 h at 37°C in a CO_2 incubator. At the end of this period, the plate was read under 405 nm wavelengths by ELISA reader (Thermo Electron Corporation Multiskan Spectrum, Vantaa, Finland). The absorbance values are normalized to protein concentrations determined by Bradford assay as described previously (10).

We have also examined the loss of mitochondrial membrane potential (MMP), another important sign of apoptosis, in response to resveratrol, C8:ceramide, PDMP, and SK-1 inhibitor or combinations of resveratrol with the others in K562 cells by the APO LOGIX JC-1 Mitochondrial Membrane Potential Detection Kit (Cell Technology, Mountain View, CA). This kit uses JC-1, a unique cationic dye, to signal the loss of the MMP. JC-1 accumulates in the mitochondria, which stains red in non-apoptotic cells, while in apoptotic cells, the MMP collapses and thus the JC-1 remains in the cytoplasm as a monomer that stains green under fluorescent light. Briefly, the cells (1×10^6 cells/2 mL), induced to undergo apoptosis, were collected by centrifugation at 1,000 rpm for 10 min. Supernatants were removed, 500 μl of JC-1 dye was added onto the pellets, and the cells were incubated at 37°C in 5% CO_2 for 15 min. Then, they were centrifuged at 1,000 rpm for 5 min, and 2 mL of assay buffer was added onto the pellets. After centrifugation at 1,000 rpm for 5 min, all pellets were resuspended with 500 μl assay buffer and 150 μl from each of them was added into the 96-well plate. The aggregate red form has absorption/emission maxima of 585/590 nm, and the monomeric green form has absorption/emission maxima of 510/527 nm. The plate was read in these wavelengths by fluorescence ELISA reader (Thermo Varioskan Spectrum, Vantaa, Finland). At the end, green/red (510/585) values were calculated to determine the changes in MMP.

In addition, K562 cells were treated with increasing concentrations of resveratrol in combinations with C8:ceramide, PDMP, or SK-1 inhibitor. For this purpose, K562 cells (1×10^6 cells/2 mL) were seeded into 6-well plates and then treated with IC50 value of C8:ceramide or IC10 values of PDMP or SK-1 inhibitor. After incubating the plates for 18 h at 37°C in 5% CO_2, increasing concentrations of resveratrol were applied into these plates. After 72-h incubation, the assay procedure mentioned above was executed.

Total RNA Isolation and RT-PCR

Total RNAs of K562 cells, treated with increasing doses of resveratrol, were extracted using a Ribolock RNA isolation kit (Macherey-Nagel, Düren, Germany) as described by the manufacturer. One μg of each total RNA was reverse transcribed using reverse transcriptase (Moroney Murine Leukemia Virus Reverse Transcriptase, Fermentas, Glenburnie, MD). After 50 min of incubation at 42°C, the reactions were stopped at 95°C for 5 min. The resulting total cDNAs were then used in PCR to measure the mRNA levels of LASS2, LASS4, LASS5, LASS6, SK1, GCS, and β-actin. The mRNA levels of β-actin were used as internal positive control. The primer sequences and PCR conditions were as follows: LASS2-F (5′-GCTGGAGATTCAC ATTTTAC-3′), LASS2-R (5′-GAAGACGATGAAGATGTT GT-3′), LASS4-F (5′-TGCTGTCCAGTTTCAACGAG-3′), LASS4-R (GAGGAAGTGTTTCTCCAGCG-3′), LASS5-F (5′-TCCTCAATGGCCTGCTGCTG-3′), LASS5-R (5′-CCCGGC AATGAAACTCACGC-3′), LASS6-F (5′-CTCCCGCACAA TGTCACCTG-3′), LASS6-R (5′-TGGCTTCTCCTGATTG CGTC-3′), SK1-F (5′-CCGACGAGGACTTTGTGCTAAT-3′), SK1-R (5′-GCCTGTCCCCCCAAAGCATAAC-3′), GCS-F (5′-ATGACAGAAAAAGTAGGCT-3′), GCS-R (5′-GGACA CCCCTGAGTGGAA-3′), β-actin-F (5′-CAGAGCAAGAGA GGCATCCT-3′), and β-actin-R (5′-TTGAAGGTCTCAAA CATGAT-3′). Two μl of the reverse transcriptase reaction product was amplified using these primers by PCR for 35 cycles, and their levels were normalized to that of β-actin as described previously (10).

After running the PCR products on agarose gel electrophoresis, quantification of expression levels of the genes were performed by Quantity One-1D-Gel-Imaging program (Biorad, Hercules, CA). The results were normalized to β-actin levels.

RESULTS

Resveratrol, C8:ceramide, PDMP, or SK-1 Inhibitor Showed a Dose-Dependent Cytotoxicity on Human K562 Chronic Myeloid Leukemia Cells

To assess antiproliferative effects of resveratrol, C8:ceramide, PDMP, or SK-1 inhibitor on human K562 cells, the cells were incubated with increasing concentrations of the agents for 72 h and XTT cell proliferation assay was conducted. The aim of application of C8:ceramide, PDMP, and SK-1 inhibitor is to see whether induction of generation or accumulation of ceramides inhibit proliferation of K562 cells. The results showed that there were dose-dependent decreases in cell proliferation in response to these chemicals as compared to untreated controls. IC50 values of resveratrol (Fig. 1A) and C8:ceramide (Fig. 1B) were calculated from cell proliferation plots and were found to be 80 and 60 μM, respectively, while IC10 values of PDMP (Fig. 1C) and SK-1 inhibitor (Fig. 1D) were 20 and 7 μM, respectively.

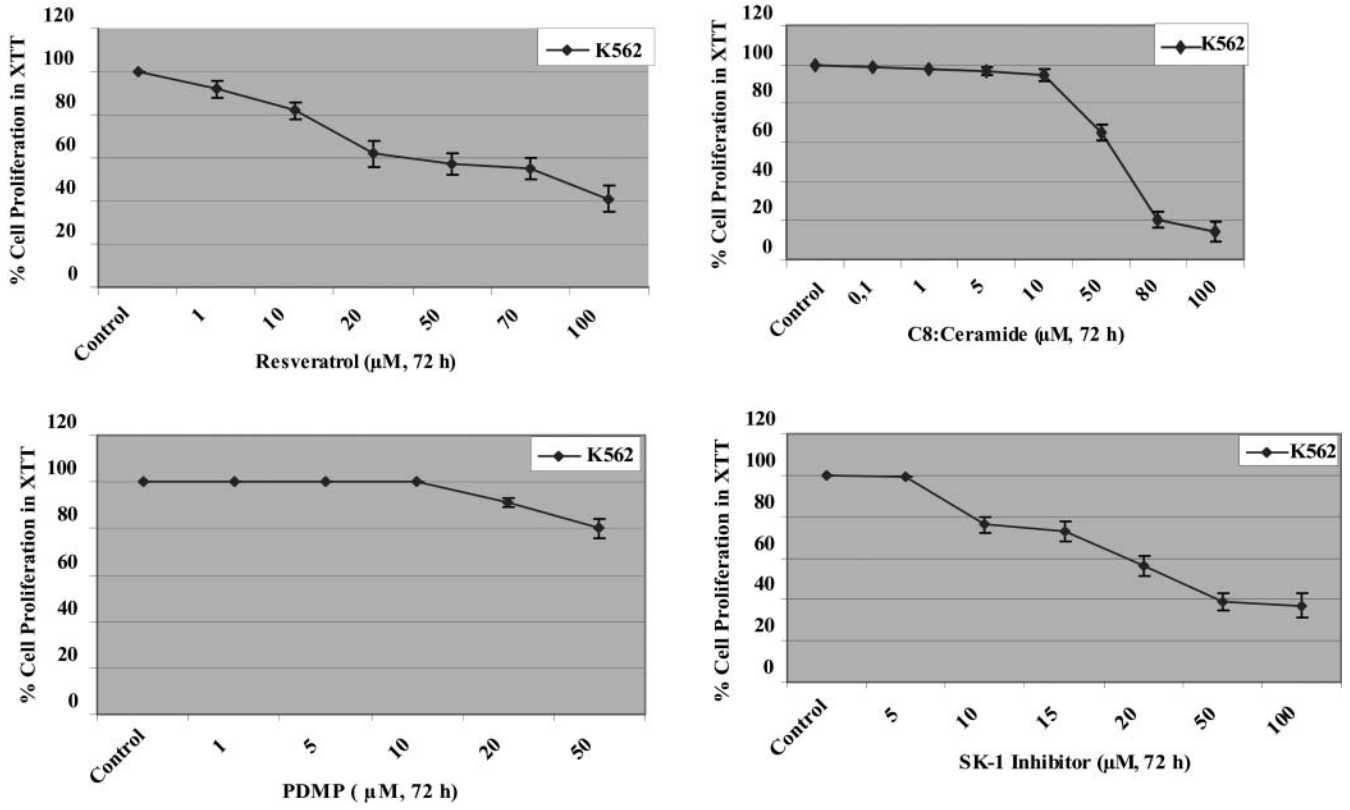

FIG. 1. Cytotoxic effects of resveratrol (A), C8:ceramide (B), PDMP (C), and SK-1 Inhibitor (D) on K562 cells. The IC50 concentration of resveratrol, C8:ceramide, and PDMP and IC10 value of SK-1 inhibitor were calculated from cell proliferation plots. The XTT assays were performed using triplicate samples in at least 3 independent experiments. The error bars represent the standard deviations.

Synergistic Cytotoxic Effects of Combinations of Resveratrol With C8:ceramide, PDMP, or SK-1 Inhibitor on K562 Cells

In order to examine the possible synergistic cytotoxic effects, the cells were exposed to increasing concentrations of resveratrol from 1 to 100 μM, together with 60 μM C8:ceramide, 20 μM PDMP, or 7 μM SK-1 inhibitor. The main purpose of these combinations was to examine if increasing intracellular concentrations of ceramides increases antiproliferative effects of resveratrol on K562 cells. Combination of 1-, 50-, and 100 μM of resveratrol with 60 μM C8:ceramide decreased proliferation of K562 cells to 87%, 88%, and 89%, respectively, while resveratrol application alone decreased cell proliferation 8%, 43%, and 59%, respectively (Fig. 2). The same doses of resveratrol in combination with 20 μM PDMP or 7 μM SK-1 inhibitor decreased cell proliferation 29%, 55%, and 68%, or 23%, 54%, and 73%, respectively (Fig. 2). The data demonstrate that increasing intracellular concentrations of ceramides increased the antiproliferative effects of resveratrol significantly. The most significant effect was observed in combination of C8:ceramide and resveratrol.

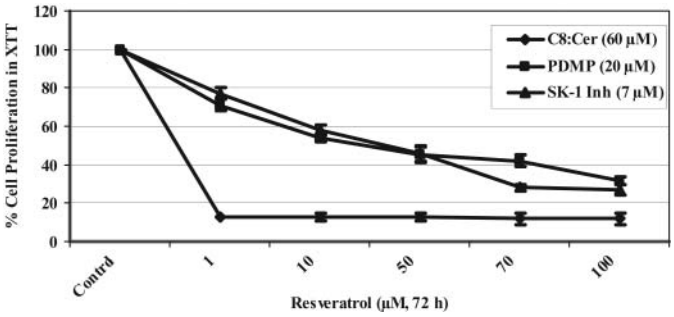

FIG. 2. Synergistic cytotoxic effects coadministration of resveratrol with C8:ceramide or PDMP or SK-1 inhibitor on K562 cells. Cytotoxicity was determined by the XTT cell proliferation assay in a 72-h culture. The XTT assays were performed using triplicate samples in at least 3 independent experiments. The error bars represent the standard deviation.

Resveratrol Together With C8:ceramide, PDMP, or SK-1 Inhibitor Induces Apoptosis Synergistically in K562 Cells

In order to see apoptotic effects of resveratrol and increased concentrations of ceramides by ceramide mimetics/inhibitors, we treated cells with different concentrations of resveratrol, C8:ceramide, PDMP, and SK-1 inhibitor alone and examined the changes in caspase-3 enzyme activity and loss of MMP. In this part of the study, we also investigated if increasing intracellular concentrations of ceramides increases

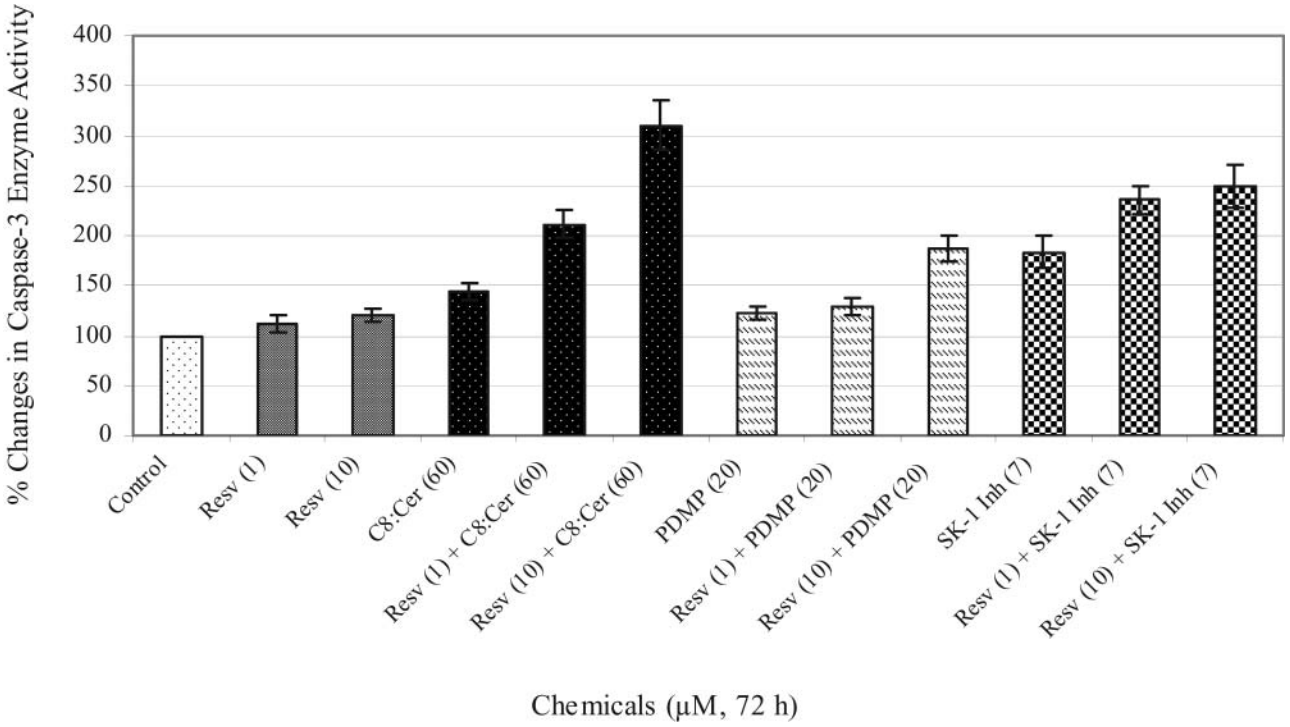

FIG. 3. Changes in caspase-3 enzyme activity in response to coadministration of resveratrol with C8:ceramide or PDMP or SK-1 inhibitor. The results are the means of 3 independent experiments. The error bars represent the standard deviations, and, when not seen, they are smaller than the thickness of the lines on the graphs.

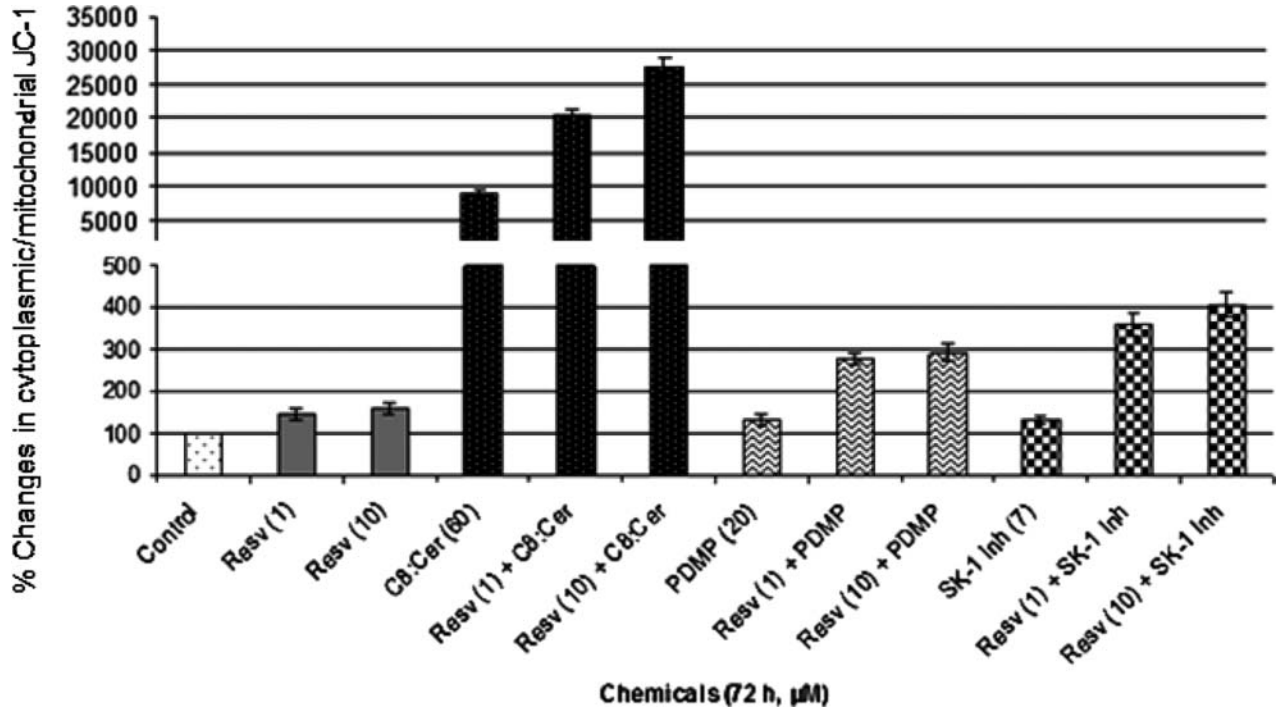

FIG. 4. Changes in cytoplasmic/mitochondrial JC-1 in K562 cells exposed to combinations of resveratrol with C8:ceramide or PDMP or SK-1 inhibitor. The results are the means of 3 independent experiments. The error bars represent the standard deviations, and, when not seen, they are smaller than the thickness of the lines on the graphs.

apoptotic effects of resveratrol. As shown in Fig. 3, there were 1.12- and 1.21-fold increases in caspase-3 enzyme activity in response to 1 and 10 μM resveratrol, respectively. Coadministration of 60 μM C8:ceramide or 20 μM PDMP or 7 μM SK-1 inhibitor with the same doses of resveratrol increased caspase-3 enzyme activity 2.11-, and 3.1- or 1.29-, and 1.87- or 2.36- and 2.49-fold, respectively. The same concentrations of C8:ceramide, PDMP and SK-1 inhibitor alone caused 1.44-, 1.22-, and 1.83-fold increases in enzyme activity, respectively.

In order to confirm caspase-3 enzyme activity results and examine the roles of mitochondria in resveratrol-induced apoptosis, we also determined the loss of mitochondrial membrane potential. The results were in agreement with our previous data and have shown that all these chemicals induce apoptosis through induction of loss of MMP. Combination of resveratrol with C8:ceramide, PDMP, or SK-1 inhibitor caused loss of MMP synergistically as compared to any agent alone and any untreated control group (Fig. 4).

Increased LASS Genes Expression and Decreased SK-1 and GCS Genes Expression Induced by Resveratrol in K562 Cells

In order to determine whether ceramide metabolizing genes are involved in resveratrol-induced apoptosis, K562 cells were treated with increasing concentrations of resveratrol (1, 10, 20, and 50 μM) and expression levels of LASS2, LASS4, LASS5, LASS6, GCS, SK-1, and ß-actin were determined by RT-PCR. As shown in Fig. 5 and Table 1, resveratrol inhibited expression levels of GCS and, more significantly, of SK-1 genes. On the contrary, expression levels of LASS2, LASS5, and to a lesser extent LASS4 and LASS6 were increased in response to resveratrol as compared to untreated controls and normalized to ß-actin levels (see Fig. 5 and Table 1).

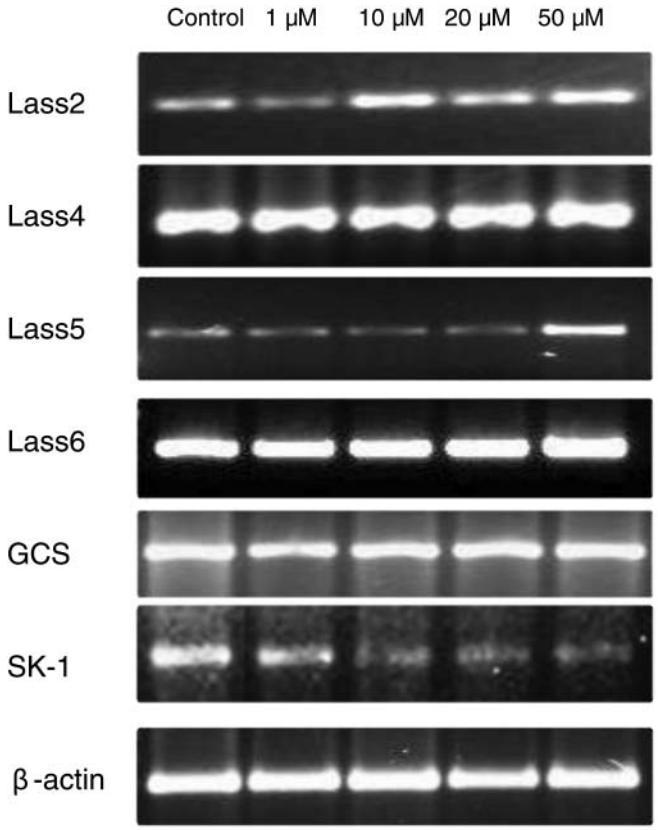

FIG. 5. Expression levels of LASS2, LASS4, LASS5, LASS6, GCS, SK-1, and β-actin genes in response to resveratrol in K562 cells. One-, 10-, 20-, and 50 μM resveratrol were applied to K562 cells for 72 h and expression levels of ceramide metabolizing genes were determined by RT-PCR. Expression levels of β-actin was detected as an internal positive control.

DISCUSSION

The incidence of cancer is increasing dramatically, and the drugs used in cancer therapy are very expensive and have many side effects. That fact opens the door to investigate novel anticancer agents originated from natural products. Resveratrol has been reported to have cytotoxic effects on various types of cancers (1). On the other hand, resveratrol has been shown to be apoptotic, autophagocytic, a radiotherapy-sensitizer, a cell cycle arrester, an inhibitor of ROS generation in mitochondria, and a regulator of p53 in many cancers (4,5,7,18–20). One of the most important properties of resveratrol is that it has selective toxic effects on cancer cells and far less effect on normal, healthy cells (21). However, the roles of bioactive sphingolipids in resveratrol-induced apoptosis and the effects of resveratrol on ceramide metabolizing genes have never before been examined in cultured chronic myeloid leukemia.

In this study, we examined potential cytotoxic and apoptotic effects of resveratrol and C8:ceramide, an important cell-permeable analogue of natural ceramides (22), which increases intracellular ceramide levels significantly, GCS and SK-1 inhibitors, which increase accumulation of ceramides on K562 cells. We treated K562 cells with increasing concentrations of these chemicals and determined their cytotoxic effects by XTT cell proliferation assay. Our results showed a dose-dependent decrease in cell proliferation in response to resveratrol and in response to increased intracellular concentrations of ceramides. Apoptosis in K562 cells exposed to increasing concentrations of these chemical agents was also evaluated by examining the changes in caspase-3 enzyme activity and the loss of the mitochondrial membrane potential. The results revealed that resveratrol, C8:ceramide, PDMP, and SK-1 inhibitor might induce apoptosis through increasing caspase-3 enzyme activity and loss of mitochondrial membrane potential. Increasing intracellular concentrations of ceramides by C8:ceramide was suggested to be an effective approach to inhibit cancer cell growth (23). Downregulation of GCS and SK-1 by biochemical and/or molecular techniques are novel effective treatment protocols to regulate cancer cell growth (24–26).

We then assessed combinational treatments of resveratrol with C8:ceramide, the inducer of de novo generation of

TABLE 1
Quantitative analyses of expression levels of GCS, SK-1, LASS2, LASS4, LASS5, and LASS6 genes in response to resveratrol

Resveratrol-Inhibited Expression Levels	GCS	SK-1	LASS2	LASS4	LASS5	LASS6
Control	100	100	100	100	100	100
1 μM Res	83	69	91	101	73	105
10 μM Res	82	41	174	102	57	109
20 μM Res	92	43	182	121	77	129
50 μM Res	83	35	191	121	188	131

apoptotic ceramides, PDMP, the inhibitor of GCS, and SK-1 inhibitor. When we coadministrate the constant concentrations of C8:ceramide (60 μM), PDMP (20 μM), or SK-1 inhibitor (7 μM) with increasing concentrations of resveratrol (1 to 100 μM), synergistic antiproliferative effects were observed as compared to any agent alone and untreated controls. Ten μM resveratrol by itself could repress only 18% of cell proliferation, while the same concentration of resveratrol, together with C8:ceramide, PDMP, or SK-1 inhibitor, inhibited 87%, 46%, and 42% of cell proliferation, respectively (Fig. 2). The synergistic apoptotic effects of resveratrol in combination with C8:ceramide, PDMP, or SK-1 inhibitor were also observed by the changes in caspase-3 enzyme activity and loss of mitochondrial membrane potential. As we increased the intracellular concentrations of ceramides by application of exogenous ceramide or by inhibition of the conversion of ceramides to glucosylceramide or sphingosine-1-phosphate, the sensitivity of K562 cells to resveratrol increased synergistically. Our results were in agreement with the literature since it was clearly shown that both caspases and mitochondrial membrane potential are well-known downstream targets of ceramides (12).

By our group and some other groups it was reported that resveratrol application results in increases in endogenous ceramide concentrations in prostate, breast (27), and colorectal carcinoma (2) and acute promyelocytic leukemia cells (28). Increases in intracellular concentrations of ceramides were shown by expression levels of the LASS gene family, GCS and SK-1 genes, and direct detection of ceramides by LC-MS. There are 6 genes in the LASS gene family that regulate de novo ceramide synthesis (29). Mammalian ceramides exist in various lengths with their different fatty acids, and each member of the LASS gene family is responsible for the generation of these different types of ceramides with different lengths (30). For instance, LASS1 gene is responsible for the synthesis of C18:ceramide (31), whereas LASS2 gene is responsible for the synthesis of C24:ceramide (32), and LASS4 gene is responsible for the synthesis of C22:ceramide (33). However, LASS5 and LASS6 genes are responsible for the synthesis of C14:ceramide and C16:ceramide (34,35). RT-PCR results of this study have shown for the first time that resveratrol treatment resulted in the upregulation of LASS2, LASS4, LASS5, and LASS6 genes in a dose-dependent fashion in human K562 CML cells. There were significant decreases in the expression levels of GCS and SK-1 genes in response to increasing concentrations of resveratrol in K562 cells. These results suggest that resveratrol increases intracellular concentrations of ceramides via induction of its de novo synthesis and also inhibition of the conversion of endogenous apoptotic ceramide to antiapoptotic glucosylceramide and sphingosine-1-phosphate molecules.

In conclusion, we confirmed the cytotoxic effects of resveratrol on K562 cells and showed that there were synergistic apoptotic effects of the combinations of resveratrol with C8:ceramide or PDMP or SK-1 inhibitor on K562 CML cells. More importantly, we have shown for the first time that resveratrol triggers apoptosis through increasing expression levels of LASS genes and inhibiting the expression levels of GCS and SK-1 in human K562 CML cell lines. Taken together, all these results may open the way toward using resveratrol and ceramides as a novel therapeutic or supportive approach for the management of CML.

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