Resveratrol Inhibits Drug-Induced Apoptosis in Human Leukemia Cells by Creating an Intracellular Milieu Nonpermissive for Death Execution

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ABSTRACT

Efficient apoptotic signaling is a function of a permissive intracellular milieu created by a decrease in the ratio of superoxide to hydrogen peroxide and cytosolic acidification. Resveratrol (RSV) triggers apoptosis in some systems and inhibits the death signal in others. In this regard, the inhibitory effect on hydrogen peroxide-induced apoptosis is attributed to its antioxidant property. We provide evidence that exposure of human leukemia cells to low concentrations of RSV (4–8 µM) inhibits caspase activation, DNA fragmentation, and translocation of cytochrome c induced by hydrogen peroxide or anticancer drugs C2, vincristine, and daunorubicin. Interestingly, at these concentrations, RSV induces an increase in intracellular superoxide and inhibits drug-induced acidification. Blocking the activation of NADPH oxidase complex neutralized RSV-induced inhibition of apoptosis. Furthermore, our results implicate intracellular hydrogen peroxide as a common effector mechanism in drug-induced apoptosis that is inhibited by preincubation with RSV. Interestingly, decreasing intracellular superoxide with the NADPH oxidase inhibitor diphenylidonium reversed the inhibitory effect of RSV on drug-induced hydrogen peroxide production. These data show that low concentrations of RSV inhibit death signaling in human leukemia cells via NADPH oxidase-dependent elevation of intracellular superoxide that blocks mitochondrial hydrogen peroxide production, thereby resulting in an intracellular environment nonconducive for death execution.

INTRODUCTION

The effector components of apoptotic death signaling and their intricate networking have been unraveled during the past couple of decades (1–3). Consequently, it is now well established that depending on the level of activation of the initiator caspase, such as caspase-8, the death signal can recruit directly downstream effector caspases or engage the mitochondria with the resultant release of effector components of apoptotic death signaling and their resultant release of death amplification factors, such as cytochrome c, apoptosis inducing factor, and Smac/DIABLO (2, 4, 5). Death signaling by anticancer drugs generally relies on positive input from the mitochondria, as is evidenced by the resistance of tumor cells overexpressing the death-inhibitory protein Bcl-2 that is localized to the membranes of mitochondria, endoplasmic reticulum, and nucleus (6–8). Therefore, by implication, an intracellular milieu permissive for caspase activation/activity and recruitment of mitochondria-derived amplification factors is critical for efficient apoptotic execution. To that end, we have demonstrated the critical role of cellular redox status in the regulation of death signaling (9–12). Whereas an overwhelming accumulation of intracellular reactive oxygen species could create an oxidatively stressed environment leading to necrosis, a slight increase is a stimulus for cellular proliferation (13, 14). Pro-oxidant intracellular milieu is a hallmark of many tumor cells and is believed to endow tumor cells with a survival advantage over their normal counterparts (15, 16).

Furthermore, we have demonstrated that a slightly elevated intracellular concentration of superoxide (O2 ·−) inhibited apoptotic signaling, irrespective of the trigger (17, 18). Contrarily, our data and that of others have highlighted the critical role of intracellular H2O2 in rendering the cytosolic milieu permissive for efficient apoptotic execution (19–21). Henceforth, we hypothesize that a critical balance between intracellular H2O2 and O2 ·− dictates the response of tumor cells to apoptotic stimuli (9, 10, 12), and any stimulus/signal that inhibits the ability of intracellular H2O2, triggered on drug exposure, to reduce the intracellular environment could potentially favor the acquisition of the resistant phenotype.

One area of recent interest in cancer biology is the chemopreventive potential of natural products. Among the compounds being evaluated for their cancer inhibiting activity is a phytoalexin, resveratrol (RSV), found in grapes and wines and known for its diverse biological activities, including antioxidant property (22–24). We reported previously that the chemopreventive activity of RSV could be the result of its ability to induce apoptotic death in human leukemia and breast carcinoma cells (25). However, depending on the cell type and the concentration used, RSV has been shown to induce or inhibit cellular proliferation and death signaling (25–29). Our present study was stimulated by a recent report that H2O2-induced apoptosis was inhibited in the presence of RSV and the implication that this could be a function of its antioxidant activity (30). Given our recent findings that drug exposure of human leukemia cells resulted in H2O2-dependent apoptosis, we set out to investigate the mechanism by which RSV inhibited apoptotic signaling triggered by exogenous H2O2 or by exposure to three anticancer agents, namely, C2, vincristine, or daunorubicin, that induce apoptotic death in cancer cells (21).

MATERIALS AND METHODS

Determination of Cell Viability and DNA Fragmentation. Human pro-myelocytic leukemia (HL60) cell line was purchased from American Type Culture Collection (Manassas, VA) and maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 1% l-glutamine, and 1% S-penicillin. In a typical survival assay, HL60 cells (1 × 105 cells/well) plated in 96-well plates were preincubated with RSV (4–8 µM) for 2 h and then treated with 100 µM of H2O2, 50 µg/ml of C2, 1.25 µg/ml of vincristine, or 0.2 µg/ml of daunorubicin for 18 h. Cell survival was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously or by the β-galactosidase (β-gal) survival assay described below (31).

Propidium iodide staining was performed for analyzing DNA fragmentation as described previously (25). Stained cells were analyzed by flow cytometry (Coulter EPICS ELITE ESP; Beckman Coulter, Fullerton, CA) with the excitation and emission wavelengths at 488 nm and 610 nm, respectively. At least 10,000 events were analyzed by WinMDI software.

Determination of Caspase-3 and -9 Activity. Caspase-3 and -9 activity was determined using the Bio-Rad fluorescent assay kit (Hercules, CA) for the two caspases. Cells (1 × 106) were preincubated for 2 h with RSV (4–8 µM) and then incubated for 12 h with 100 µM H2O2, 50 µg/ml of C2, 1.25 µg/ml of vincristine, or 0.2 µg/ml of daunorubicin. Fifty µl of 2X reaction buffer with 10 mM DTT and 5 µl of the conjugate substrate (DEVD-AFC for caspase-3 and LEHD-AFC for caspase-9) were added to cell lysates. Caspase activity was determined by the relative fluorescence intensity at 505 nm following excitation at 400 nm with a spectrophluorometer (Tecan Group, Ltd., Maennedorf, Switzerland). Results are shown as fold increase (X-increase) in

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1452
activity relative to untreated cells (1×). In addition, cleavage of the caspase-3 substrate, poly(ADP-ribose) polymerase (PARP), was assessed by Western blot analysis using anti-PARP (clone C2-10; PharMingen, San Diego, CA) as described previously (9).

Measurements of Intracellular O$_2^-$ and pH. Intracellular O$_2^-$ was assayed by a lucigenin-based chemiluminescence assay as described previously (11). Data are shown as percentage change (% change) in the intracellular O$_2^-$ concentration compared with untreated cells and are the mean ± SD of three independent measurements.

For measurement of cytosolic pH, cells were loaded with 10 μM 2′,7′-bis(2-carboxyethyl)-5,6-carboxyfluorescein diacetate (BCECF-AM; Sigma, St. Louis, MO), and the fluorescence ratio of 525:610 nm was used to derive cytosolic pH using a standard pH calibration curve as described previously (11).

Flow Cytometric Analysis of Intracellular H$_2$O$_2$. Cells were exposed to the apoptotic triggers for 4–12 h, loaded with 5-(and-6)-chloromethyl-2′,7′-dichlorofluorescin diacetate (CM-H$_2$DCFDA; Molecular Probes, Eugene, OR; 5 μM) (32) at 37°C for 15 min, and analyzed by flow cytometry (Coulter EPICS Elite ESP) using an excitation wavelength of 488 nm as described previously (21). At least 10,000 events were analyzed.

Determination of Mitochondrial ΔΨ$_{m}$ and Cytosolic Cytochrome c. Potential-sensitive probe 3,3′dihexyloxacarbocyanine iodide was used to measure mitochondrial ΔΨ$_{m}$, as described previously (31). Briefly, 1 × 10$^6$ cells were incubated with 3,3′dihexyloxacarbocyanine iodide (40 nm) for 15 min at 37°C. As a positive control, cells were incubated separately with an uncoupling agent, carbonyl cyanide m-chlorophenylhydrazone (CCCP) (100 μM). At least 10,000 events were analyzed by flow cytometry with excitation set at 488 nm.

Cytosolic c was detected in cytosolic extracts from 30 × 10$^6$ cells by Western blot analysis using anti-cytochrome c (7H8.2C12; PharMingen) as described previously (33).

Transient Transfection with pIRESRacN17 and β-Gal Survival Assay. Transient transfections of CEM cells were performed using the SuperFect transfection reagents from Qiagen (Hilden, Germany), and survival of transfected cells was assessed by the β-gal survival assay as described recently (34). Cell survival was calculated as [(β-gal activity μg$^{-1}$ protein of transfected cells incubated with the apoptotic trigger)/(β-gal activity μg$^{-1}$ protein of transfected cells incubated without the trigger)]. β-Gal activity was measured using the Galacto-Star mammalian reporter kit (Applied Biosystems, Foster City, CA). Protein concentration was determined using the Coomasie Plus protein assay reagent from Pierce (Rockford, IL).

**RESULTS**

Low Doses of RSV Inhibit H$_2$O$_2$-Induced Apoptosis Upstream of the Mitochondria. Corroborating earlier findings on the death-inducing activity of H$_2$O$_2$ in cancer cells, exposure of HL60 cells to H$_2$O$_2$ (100 μM) resulted in a significant decrease in cell survival (Fig. 1A). The cytotoxic activity of H$_2$O$_2$ was a function of activation of the apoptotic death pathway as evidenced by the significant increase in caspase activity, cleavage of the caspase-3 substrate PARP, and appearance of the sub-G1 fraction (Fig. 1, B–D). Interestingly, preincubation of cells with 4–8 μM RSV for 2 h before the addition of H$_2$O$_2$ for 18 h resulted in an increase in cell survival (Fig. 1A), significant inhibition of caspase-3 activity and PARP cleavage (Fig. 1, B and C), and inhibition of DNA fragmentation (Fig. 1D). Moreover, preincubution with RSV prevented H$_2$O$_2$-induced decrease in mitochondrial ΔΨ$_{m}$ (Fig. 2A) and significantly blocked H$_2$O$_2$-induced cytosolic translocation of cytochrome c from the mitochondria (Fig. 2B). One possible explanation for the observed inhibitory effect of RSV could be that RSV functioned as an efficient scavenger of H$_2$O$_2$. However, addition of RSV and H$_2$O$_2$ simultaneously to the culture medium (RSV/H$_2$O$_2$) had no effect on H$_2$O$_2$-induced cell death (Fig. 2C), whereas previous incubation with RSV for 2 h (RSV + H$_2$O$_2$) significantly (P < 0.05) inhibited apoptotic signaling (Fig. 2C). These data indicate that the death-inhibitory effect of RSV is not simply a function of its ability to scavenge H$_2$O$_2$ but involves mechanism(s) upstream of the mitochondria or signals that engage the mitochondrial death machinery.

**RSV Inhibits H$_2$O$_2$-Induced Decreases in Intracellular O$_2^-$ and Cytosolic pH.** We have shown that H$_2$O$_2$ induces a decrease in O$_2^-$ and cytosolic acidification, thereby creating an intracellular milieu permissive for apoptotic execution (18, 34, 35). Therefore, we questioned if the inhibitory effect on H$_2$O$_2$-induced apoptosis was caused by the ability of RSV to create an intracellular environment noncon-

![Fig. 1](https://example.com/fig1.png)

**Fig. 1.** Preincubation with resveratrol (RSV) inhibits H$_2$O$_2$-induced caspase activation and DNA fragmentation in HL60 cells. A, HL60 cells (1 × 10$^6$ cell/ml) were incubated with 100 μM H$_2$O$_2$ for 18 h with or without preincubation for 2 h with RSV (4 and 8 μM). Cell viability was determined by the MTT assay. B, caspase-9 and -3 activity was determined by fluorometric assays, and C, poly(ADP-ribose) polymerase cleavage was assessed by Western blot analysis. D, DNA fragmentation was determined by propidium iodide staining and the appearance of sub-G1 fraction.

**Data Analysis.** Data presented are mean ± SD of at least three independent experiments performed in triplicate, unless otherwise indicated. Statistical significance was determined by the Student’s t test.
Inhibitory effect of resveratrol (RSV) on H₂O₂-induced death signaling is upstream of the mitochondria. A, HL60 (1 × 10⁶) cells were exposed to 100 µM H₂O₂ for 4 h with or without previous incubation for 2 h with RSV (8 µM), and ΔΔh₉₆₀ was determined as described in "Materials and Methods." R₉, cytosolic cytochrome c was assessed by Western blot analysis. Staurosporin-treated HL60 cytosolic extract was used as a positive control. C, HL60 cells (1 × 10⁶/ml) were incubated simultaneously with RSV (8 µM) and H₂O₂ (100 µM) for 18 h (RSV/H₂O₂) or preincubated for 2 h with RSV (8 µM) before the addition of 100 µM H₂O₂ (RSV + H₂O₂). Cell viability was determined by MTT assay.

RSV Induced Inhibition of Apoptosis Can Be Reverted by Blocking NADPH Oxidase Activation. Considering the reports on the antioxidant potential of polyphenolic compounds, such as RSV, we were intrigued by our observation that low concentrations of RSV induced an increase, rather than a decrease, in intracellular O₂⁻ concentration (30, 36–38). Therefore, we investigated the effect of blocking NADPH oxidase complex, one of the major sources of intracellular O₂⁻, on RSV-induced increase in O₂⁻. Pharmacologic inhibition of NADPH oxidase with diphenyleneiodonium chloride (DPI) completely blocked RSV-induced increase in intracellular O₂⁻ in HL60 cells (Fig. 3C). Furthermore, incubation of cells for 1 h with DPI before the addition of RSV significantly (P < 0.004) restored the sensitivity of HL60 cells to H₂O₂, which was even more pronounced on subsequent addition of C2 (Fig. 3D). It should be noted that a slight increase in intracellular O₂⁻ also was observed on exposure of cells to C2 alone; however, unlike RSV, this was accompanied by a surge in intracellular H₂O₂ production. In addition, cytosolic acidification triggered on exposure to C2 (Fig. 4A) resulted in a decrease of intracellular O₂⁻, whereas simultaneous exposure to RSV and C2 (RSV/C2) had no effect on C2 signaling (data not shown).

We next questioned whether the inhibitory effect of RSV on C2-induced apoptosis also was mediated by its ability to (a) create a pro-oxidant intracellular milieu; and (b) inhibit cytosolic acidification. Results indicate that preincubation of HL60 cells with RSV inhibited H₂O₂ production triggered by exposure to C2 (Fig. 5A). Similar to the results obtained with H₂O₂, preincubation of HL60 cells with RSV for 2 h resulted in an increase in intracellular O₂⁻ concentration, which was more pronounced on subsequent addition of C2 (Fig. 5B). It should be noted that a slight increase in intracellular O₂⁻ also was observed on exposure of cells to C2 alone; however, unlike RSV, this was accompanied by a surge in intracellular H₂O₂ production. In addition, cytosolic acidification triggered on exposure to C2 was inhibited completely on previous exposure to RSV (Fig. 5C). Furthermore, incubation of cells with DPI before the addition of RSV and C2 resulted in a decrease in intracellular O₂⁻ (data not shown) and restored death signaling in response to C2 (Fig. 5D).

To gain additional insight into the death-inhibitory effect of RSV and its potential clinical implications, we next investigated the effect of RSV on apoptosis induced by two chemotherapeutic agents, vincristine and daunorubicin (39). Corroborating the results obtained with H₂O₂ and C2, results indicate a dose-dependent (up to 8 µM) inhibitory effect of RSV on vincristine-induced cell death (Fig. 6A) together with significant inhibition of caspase-9 and -3 activity (Fig. 6B). That the apoptosis inhibitory effect of RSV was linked to its ability to create a pro-oxidant intracellular milieu was supported additionally by the ability of DPI or transient transfection with RacN17 to revert the sensitivity of leukemia cells in the presence of RSV to vincristine-induced apoptosis (Fig. 6, C and D).

The inhibitory effect of low concentrations of RSV on drug-induced apoptosis was not exclusive to C2 or vincristine. Preincubation of leukemia cells to RSV for 2 h resulted in a significant increase in cell survival (Fig. 7A), inhibition of caspase activity (Fig. 7B), and inhibition of intracellular H₂O₂ production (Fig. 7C) induced by exposure to daunorubicin.
Fig. 3. Inhibition of NADPH oxidase activation prevents resveratrol (RSV)-induced increase in intracellular $O_2^-$ and overcomes its death-inhibitory effect. A, cytosolic pH was determined with the pH-sensitive probe 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein. B, $2 \times 10^6$ cells were treated with RSV (8 μM) for 2 h or $H_2O_2$ (100 μM) for 4 h with or without 2 h of previous incubation with RSV (8 μM). Intracellular $O_2^-$ was measured by a lucigenin-based chemiluminescence assay. C, HL60 ($2 \times 10^6$) cells were incubated with 8 μM RSV for 4 h in the presence or absence of DPI (1.25 μM), and intracellular $O_2^-$ was measured as described previously. D, HL60 cells ($1 \times 10^6$/ml) were preincubated with DPI (1.25 μM) or with DPI (1.25 μM) + RSV (8 μM) for 4 h before the addition of 100 μM $H_2O_2$ for 18 h. Cell survival was assessed by the MTT assay. E, CEM cells ($1 \times 10^6$/ml) cotransfected with pCMV-β-galactosidase (β-gal) and pIRES-RacN17 were exposed to 100 μM $H_2O_2$ for 18 h with or without previous incubation for 2 h with 8 μM RSV. Cell survival was assessed by the β-gal survival assay. Mean of two independent transfections done in duplicate is shown.
NADPH oxidase complex had no effect on intracellular H$_2$O$_2$ increase (21). Similar to the results reported with C2, preincubation of HL60 cells with DPI did not affect intracellular H$_2$O$_2$ production on exposure to vincristine and daunorubicin, thus strongly suggesting mitochondria as the cellular source on drug exposure (data not shown). More importantly, decreasing intracellular O$_2^-$ with DPI restored the ability of C2, vincristine, and daunorubicin to trigger intracellular H$_2$O$_2$ production even in the presence of RSV (Fig. 7C).

**DISCUSSION**

**RSV Inhibits Apoptosis by Altering Cellular Redox Status.** Taken together, our data provide strong evidence that contrary to its proapoptotic activity at $\geq 25$ μM, low micromolar concentrations (4–8 μM) inhibit apoptotic signaling (25, 40, 41). This divergent signaling by RSV is intriguing and could be explained by our earlier observations that at concentrations of $\geq 32$ μM, apoptosis induced in HL60 cells is mediated by up-regulation of CD95 (Fas/Apo1)-CD95L interaction (25). This effect on up-regulation of the death receptor ligand is not observed at concentrations of RSV <16 μM, hence the inability to trigger apoptosis in these cells (data not shown). Contrarily, at these concentrations, RSV inhibits apoptotic signaling upstream of the mitochondria, thus blocking the recruitment of mitochondrial-derived amplification factors, such as cytochrome c. We also provide evidence that RSV has a potent effect on the intracellular redox status, a critical determinant of the efficacy of the death signal (9, 42, 43). In that respect, it has been shown previously that maintaining a slightly elevated intracellular O$_2^-$ promotes cellular proliferation (14, 44) and inhibits apoptotic signaling (18, 42). A pro-oxidant intracellular milieu is an invariable finding in cancer cells and has been shown to endow cancer cells with a survival advantage over their normal counterparts (15). Because a decrease in intracellular O$_2^-$ and cytosolic pH is critical for efficient death execution, our results suggest that low concentrations of RSV could enable cancer cells to evade death signaling by creating a pro-oxidant intracellular milieu and inhibiting cytosolic acidification.

**RSV Induces Increase in O$_2^-$ via NADPH Oxidase Activation.** Considering the earlier reported ability of RSV to inhibit mitochondrial complex III-induced reactive oxygen species production, our paradoxical findings provide evidence for a pro-oxidant effect of RSV at concentrations that do not trigger apoptosis (45). Such pro-oxidant activity of polyphenolics, such as RSV, has been reported recently in different systems (46, 47). In one model, reactive oxygen species generation at concentrations of RSV that triggered cell death in human cancer cells was proposed to be responsible for its cytotoxic activity (47). In addition, our results implicate the membrane NADPH oxidase complex as a potential source of O$_2^-$ on incubation with low doses of RSV. Inhibition of the NADPH oxidase complex not only restored death signaling but also resulted in reverting the negative effect of RSV on drug-induced intracellular H$_2$O$_2$ production. This fits in well with our hypothesis that a balance between intracellular O$_2^-$ and H$_2$O$_2$ could be a critical factor in the response of cells to apoptotic triggers, with a tilt toward the former favoring survival and a predominance of the latter facilitating death execution (10). A careful look at the data shown in Fig. 5B reveals that, similar to RSV alone, exposure of cells to the anticancer drug C2 also results in a slight elevation in intracellular O$_2^-$ level. However, it is important to note that C2-induced O$_2^-$ is accompanied by a surge in intracellular H$_2$O$_2$, which is not observed with low concentrations of RSV. In addition, preincubation with RSV completely blocks C2-induced H$_2$O$_2$ production and maintains a significantly elevated intracellular O$_2^-$ level. By implication, this suggests that an increase in intracellular O$_2^-$ could inhibit downstream H$_2$O$_2$ production from the mitochondria, thereby
inhibiting the decrease in intracellular pH. It is intriguing as to how an increase in intracellular O$_2^-$ blocks mitochondrial-derived H$_2$O$_2$. One possibility could be inhibition of upstream caspase activation, as has been shown previously, or other proapoptotic factors, such as death-promoting Bcl-2 family members, required for the engagement of the mitochondrial pathway (48). Impeding these upstream pathways could account for the inhibition of drug-induced mitochondrial H$_2$O$_2$ production and downstream cytochrome c release observed in cells preincubated with RSV. This in turn fails to amplify downstream caspase cascade (caspase-9 and -3) and leads to a substantial decrease in the sensitivity of cells to apoptotic stimuli that require mitochondrial amplification factors. Taken together, these results indicate that the increase in intracellular O$_2^-$ on exposure of leukemia cells to RSV was mediated through the activity of NADPH oxidase complex and linked directly or indirectly to the apoptosis-inhibitory activity of RSV. In addition, data presented here not only implicate mitochondrial H$_2$O$_2$ production as a critical effector mechanism during drug-induced apoptosis but also demonstrate the ability of an increase in intracellular O$_2^-$ to prevent H$_2$O$_2$ production and thereby impede the recruitment of the mitochondrial death pathway.

Fig. 5. Resveratrol (RSV) creates a pro-oxidant intracellular milieu and inhibits C2-mediated decrease in cytosolic pH and cell death. A, HL60 (1×10$^6$) cells were treated with 50 μg/ml of C2 for 4 h with or without previous incubation with 8 μM RSV, and intracellular H$_2$O$_2$ was determined. B, 2×10$^6$ cells (in 2 ml) were treated with RSV (8 μM) for 4 h or 50 μg/ml of C2 for 4 h with or without 2 h of previous incubation with RSV (8 μM), and intracellular O$_2^-$ was determined. C, cells were treated as in (A), and cytosolic pH was assessed with 2',7'-bis(2-carboxyethyl)-5,6-carboxyfluorescein. D, HL60 cells (1×10$^6$/ml) were preincubated with DPI (1.25 μM) or with DPI (1.25 μM) + RSV (8 μM) for 4 h before the addition of 50 μg/ml C2 for 18 h. Cell survival was assessed by the MTT assay.

Fig. 6. The inhibitory effect of resveratrol (RSV) on vincristine-induced apoptosis can be neutralized by inhibition of the NADPH oxidase complex. A, HL60 cells (1×10$^6$/cell/ml) were preincubated with RSV (2, 4, or 8 μM) for 2 h before treatment with 1.25 μg/ml of vincristine for 18 h. Cell viability was determined by the MTT assay. B, caspase-9 and -3 activity was determined by fluorimetric assays. C, HL60 cells (1×10$^6$/ml) were preincubated with DPI (1.25 μM or 2.5 μM) + RSV (8 μM) for 4 h before the addition of 1.25 μg/ml of vincristine for 18 h. Cell survival was assessed by the MTT assay. D, CEM leukemia cells (1×10$^6$/ml) were cotransfected with pCMV-β-galactosidase (β-gal) and pIRES-RacN17 and exposed to 1.25 μg/ml of vincristine for 18 h with or without previous incubation for 2 h with 8 μM RSV. Cell survival was assessed by the β-gal assay.
Fig. 7. Resveratrol (RSV) inhibits daunorubicin-induced apoptosis, and preincubation with DPI reverses the effect of RSV on drug-induced H₂O₂ production. A, 1 × 10⁵ cell/ml were incubated with RSV (8 μM) for 2 h, followed by 18 h of exposure to 0.2 μg/ml of daunorubicin. Cell viability was determined by the MTT assay. B, caspase-9 and -3 activity was determined by fluorimetric assays. C, HL60 (1 × 10⁵) cells were preincubated with 1.25 μM DPI before the addition of RSV (DPI/RSV), followed by treatment with C2 (50 μg/ml), daunorubicin (0.2 μg/ml), or vincristine (1.25 μg/ml) for 4 h. Intracellular H₂O₂ was determined by flow cytometry.

Potential Implications. These findings could be potentially important in light of the recent interest in the biological activity of flavonoids or flavonoid-like molecules, such as RSV, for their possible use in combination chemotherapy regimens. Although in vitro exposure of tumor cells to RSV at relatively high concentrations results in apoptotic cell death, because of the low bioavailability of RSV, plasma levels as high as 50–100 μM may not be physiologically attainable (49, 50). Our data suggest death-inhibitory and/or prosurvival activity of RSV in leukemia cells at doses that may be relevant physiologically (49, 50). Thus, the use of RSV in combination with drugs such as C2, vincristine, or daunorubicin could be a dangerous mixture because the slight pro-oxidant effect may provide tumor cells with not only a survival advantage but also impede death signals. This could present an ideal environment for the propagation and proliferation of tumor cells.

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REFERENCES


Correction: Resveratrol Inhibits Drug-Induced Apoptosis in Human Leukemia Cells by Creating an Intracellular Milieu Nonpermissive for Death Execution

In this article (Cancer Res 2004;64:1452–9), which was published in the February 15, 2004, issue of Cancer Research (1), the authors did not cite a previously published poster presented at a symposium on “Apoptosis: From Signaling Pathways to Therapeutic Tools,” held in Luxembourg in January 2003 and published as conference proceedings (2), even though parts of the text were similar. Figures 1 and 3 from the previous article were also reused in the Cancer Research article. To correct the academic record, reference is now given to that prior publication.

References

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