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 diphenyleneiodonium 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 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). Thenceforth, 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 promyelocytic 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 × 106 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 2× reaction buffer with 10 mM DTT and 5 μl of the conjugate substrate (DEVdAFC 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 spectrofluorometer (Tecan Group, Ltd., Maennedorf, Switzerland). Results are shown as fold increase (X-fold) in
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 C-2; PharMingen, San Diego, CA) as
described previously (9).

Measurements of Intracellular O$_2^-$ and pH. Intracellular O$_2^-$ was as-
sayed 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 (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′-dihexyloxycarbocyanine iodide was used to
measure mitochondrial ΔΨ$_m$, as described previously (31). Briefly, 1 × 10$^6$
cells were incubated with 3,3′-dihexyloxycarbocyanine 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 pIRESVacN17 and β-Gal Survival Assay.
Transient transfections of CEM cells were performed using the SuperFect
transfection reagents from Qiagen (Hilden, Germany), and survival of trans-
fected 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 Coomassie Plus
protein assay reagent from Pierce (Rockford, IL).

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.

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-G$_1$ fraction (Fig. 1, B–D). Interestingly, prein-
cubation 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,
preen incubation with RSV prevented H$_2$O$_2$-induced decrease in mito-
chondrial 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 func-
tion 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 ques-
tioned if the inhibitory effect on H$_2$O$_2$-induced apoptosis was caused by
the ability of RSV to create an intracellular environment noncon-

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Fig. 1. Preincubation with resveratrol (RSV) in-
hbits 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 deter-
mained 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 frag-
mentation was determined by propidium iodide
staining and the appearance of sub-G$_1$ fraction.

Fig. 2. Preincubation with resveratrol (RSV) in-
hbits H$_2$O$_2$-induced caspase activation and DNA
fragmentation in HL60 cells.
treated HL60 cytosolic extract was used as positive control. A, HL60 cells (1 × 10⁵) 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 and CEM leukemia cells to H₂O₂ (Fig. 3D; data not shown). To provide additional evidence that this was a function of NADPH oxidase activation, CEM cells were transfected transiently with a dominant negative mutant of Rac (RacN17), which inhibits NADPH oxidase-dependent increase in intracellular O₂⁻ (34). Similar to DPI, transient transfection with RacN17 completely neutralized the death-inhibitory activity of RSV and restored the sensitivity of leukemia cells to H₂O₂ (Fig. 3E).

RSV Inhibits Apoptosis Triggered by Anticancer Drugs C2, Vincristine, and Daunorubicin. In our earlier reports, we demonstrated that exposure of human leukemia and melanoma cells to C2, a purified photoprodut of MC540, triggered mitochondrial generation of H₂O₂ that was responsible for the release of cytochrome c and downstream activation of the caspase cascade (21). Similar to the data obtained with H₂O₂, preincubation of cells with RSV before the addition of C2 resulted in an increase in cell survival compared with the cells treated with C2 alone (Fig. 4A). Whereas exposure of leukemia cells to C2 resulted in robust increases in caspase-3 and -9 activity, preincubation with RSV significantly inhibited both caspases and the cleavage of the caspase-3 substrate PARP (Fig. 4B). That this inhibition was mediated via blocking mitochondria-dependent death signaling was evidenced by the significant decrease in cytosolic translocation of cytochrome c in cells preincubated with RSV before the addition of C2 (Fig. 4C). In addition, similar to the results obtained with H₂O₂, previous incubation with RSV (RSV + C2) was required for the inhibitory effect of RSV on C2-induced death signaling, 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 even 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 C2 was inhibited completely on previous exposure to RSV (Fig. 5D). Similar to DPI, transient transfection with RacN17 completely neutralized the death-inhibitory activity of RSV (Fig. 5E).

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 $\text{O}_2^-$ and overcomes its death-inhibitory effect. 

A, cytosolic pH was determined with the pH-sensitive probe 2',7'-bis(2-carboxylethyl)-5,6-carboxyfluorescein. 

B, $2 \times 10^6$ cells were treated with RSV (8 $\mu$M) for 2 h or $\text{H}_2\text{O}_2$ (100 $\mu$M) for 4 h with or without 2 h of previous incubation with RSV (8 $\mu$M). Intracellular $\text{O}_2^-$ was measured by a lucigenin-based chemiluminescence assay. 

C, HL60 ($2 \times 10^6$) cells were incubated with 8 $\mu$M RSV for 4 h in the presence or absence of DPI (1.25 $\mu$M), and intracellular $\text{O}_2^-$ was measured as described previously. 

D, HL60 cells ($1 \times 10^6$/ml) were preincubated with DPI (1.25 $\mu$M) or with DPI (1.25 $\mu$M) + RSV (8 $\mu$M) for 4 h before the addition of 100 $\mu$M $\text{H}_2\text{O}_2$ 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 $\mu$M $\text{H}_2\text{O}_2$ for 18 h with or without previous incubation for 2 h with 8 $\mu$M RSV. Cell survival was assessed by the β-gal survival assay. Mean of two independent transfections done in duplicate is shown.
RSV Inhibits Apoptosis by Altering Cellular Redox Status.

Taken together, our data provide strong evidence that contrary to its proapoptotic activity at \( \geq 25 \mu M \), low micromolar concentrations (4–8 \( \mu M \)) 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 \( \geq 32 \mu M \), 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 \( \mu M \), 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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (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 the inhibitory activity of caspase-3 \( \alpha \)-chain (47).
inhibiting the decrease in intracellular pH. It is intriguing as to how an increase in intracellular \( \text{O}_2^- \) blocks mitochondrial-derived \( \text{H}_2\text{O}_2 \). 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 \( \text{H}_2\text{O}_2 \) 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 \( \text{O}_2^- \) 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 \( \text{H}_2\text{O}_2 \) production as a critical effector mechanism during drug-induced apoptosis but also demonstrate the ability of an increase in intracellular \( \text{O}_2^- \) to prevent \( \text{H}_2\text{O}_2 \) 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 \( \times \) 10\(^6\) cells were treated with 50 \( \mu \)g/ml of C2 for 4 h with or without previous incubation with 8 \( \mu \)M RSV, and intracellular \( \text{H}_2\text{O}_2 \) was determined. B, 2 \( \times \) 10\(^6\) cells (in 2 ml) were treated with RSV (8 \( \mu \)M) for 4 h or 50 \( \mu \)g/ml of C2 for 4 h with or without 2 h of previous incubation with RSV (8 \( \mu \)M), and intracellular \( \text{O}_2^- \) was determined. C, cells were treated as in (A), and cytosolic pH was assessed with 2',7'-bis(2-carboxyethyl)-5,6-carboxy-fluorescein. D, HL60 cells (1 \( \times \) 10\(^6\)/ml) were preincubated with DPI (1.25 \( \mu \)M) or with DPI (1.25 \( \mu \)M) + RSV (8 \( \mu \)M) for 4 h before the addition of 50 \( \mu \)g/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 \( \times \) 10\(^6\) cell/ml) were preincubated with RSV (2, 4, or 8 \( \mu \)M) for 2 h before treatment with 1.25 \( \mu \)g/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 \( \times \) 10\(^6\)/ml) were preincubated with DPI (1.25 \( \mu \)M or 2.5 \( \mu \)M) + RSV (8 \( \mu \)M) for 4 h before the addition of 1.25 \( \mu \)g/ml of vincristine for 18 h. Cell survival was assessed by the MTT assay. D, CEM leukemia cells (1 \( \times \) 10\(^6\)/ml) were cotransfected with pCMV-\(\beta\)-galactosidase (\(\beta\)-gal) and pIRES-RacN17 and exposed to 1.25 \( \mu \)g/ml of vincristine for 18 h with or without previous incubation for 2 h with 8 \( \mu \)M RSV. Cell survival was assessed by the \(\beta\)-gal assay.
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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