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Resveratrol increases vascular oxidative stress resistance

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Ungvari Z, Orosz Z, Rivera A, Labinskyy N, Xiangmin Z, Olson S, Podlutsky A, Csiszar A. Resveratrol increases vascular oxidative stress resistance. *Am J Physiol Heart Circ Physiol* 292: H2417–H2424, 2007. First published January 12, 2007; doi:10.1152/ajpheart.01258.2006.—Epidemiological studies suggest that Mediterranean diets rich in resveratrol are associated with reduced risk of coronary artery disease. However, the mechanisms by which resveratrol exerts its vasculoprotective effects are not completely understood. Because oxidative stress and endothelial cell injury play a critical role in vascular aging and atherogenesis, we evaluated whether resveratrol inhibits oxidative stress-induced endothelial apoptosis. We found that oxidized LDL and TNF-α elicited significant increases in caspase-3/7 activity in endothelial cells and cultured rat aortas, which were prevented by resveratrol pretreatment (10^{-6}–10^{-4} mol/l). The protective effect of resveratrol was attenuated by inhibition of glutathione peroxidase and heme oxygenase-1, suggesting a role for antioxidant systems in the antiapoptotic action of resveratrol. Indeed, resveratrol treatment protected cultured aortic segments and/or endothelial cells against increases in intracellular H_2O_2 levels and H_2O_2-mediated apoptotic cell death induced by oxidative stressors (exogenous H_2O_2, paraquat, and UV light). Resveratrol treatment also attenuated UV-induced DNA damage (comet assay). Resveratrol treatment upregulated the expression of glutathione peroxidase, catalase, and heme oxygenase-1 in cultured arteries, whereas it had no significant effect on the expression of SOD isoforms. Resveratrol also effectively scavenged H_2O_2 in vitro. Thus resveratrol seems to increase vascular oxidative stress resistance by scavenging H_2O_2 and preventing oxidative stress-induced endothelial cell death. We propose that the antioxidant and antiapoptotic effects of resveratrol, together with its previously described anti-inflammatory actions, are responsible, at least in part, for its cardioprotective effects.

endothelial cell; comet assay; caloric restriction mimetics; apoptosis; polyphenol; heme oxygenase antioxidant

EPIDEMIOLOGICAL STUDIES have shown that, in southern France and other Mediterranean territories, the morbidity and mortality of coronary artery disease is low, despite a diet rich in saturated fats and smoking habits (17, 30). This unexpected epidemiological finding was termed the "French paradox." It has been proposed that resveratrol, an important constituent of Mediterranean diets, is involved in vasculoprotection. Resveratrol has been identified in more than 70 species of plants, including grapevines (*Vitis vinifera*), mulberries (*Morus rubra*), *Vaccinum* species, and peanuts (*Arachis hypogea*), and it is thought to have diverse antiatherogenic activities (42, 55–57, 60, 61), such as the inhibition of LDL oxidation (22) and platelet aggregation (46) and regulation of vascular smooth muscle proliferation (24–26, 54). Recently, studies from this and other laboratories have shown that resveratrol inhibits endothelial activation and monocyte adhesion (12, 21, 39) and attenuates proinflammatory gene expression by inhibition of NF-κB activation in coronary arterial endothelial cells (12).

There is overwhelming evidence that oxidative stress plays a crucial role in the development of vascular disease and vascular aging (11, 13, 35). The mechanisms by which oxidative stress elicits endothelial cell injury during atherogenesis are likely to include induction of inflammatory processes (11) and activation of cellular apoptotic pathways (9). Although there is evidence for the inhibitory effect of resveratrol on oxidative stress-induced vascular inflammation (12, 35), it is not well understood how resveratrol affects cellular resistance against proapoptotic effects of oxidative stressors. Recently, the view emerged that resveratrol may induce a dose- and cell type-dependent induction of both anti- and proapoptotic mechanisms (3, 4). In cancer cells, resveratrol appears to induce programmed cell death by activating death receptors (18) and mitochondrial pathways (19), by inducing cell cycle arrest and/or by sensitizing the cells to drug-induced apoptosis (29). These observations lead to the use of resveratrol as a chemotherapeutic agent (23). In contrast, there are also studies showing that in certain malignant cells and other noncancerous cell types, resveratrol may inhibit caspase activation and attenuate apoptotic cell death (3, 4, 42). Interestingly, resveratrol was shown to inhibit apoptotic neuronal cell death induced by oxidized LDL (ox-LDL) (20). It is significant that, in various cell types, resveratrol may exert antioxidant effects, including the scavenging of reactive oxygen species (ROS) (36). However, the effects of resveratrol on vascular production of ROS have not yet been well characterized.

On the basis of the aforementioned findings, we hypothesized that resveratrol may exert antioxidant effects in the vascular tissue and attenuate oxidative stress-induced apoptosis of endothelial cells, at least in part, by decreasing ROS levels. To test these hypotheses, we determined whether in cultured rat arteries and cultured endothelial cells resveratrol inhibits induction of apoptosis by oxidative stress. For oxidative stressors we have used ox-LDL (27), exogenous H_2O_2 treatment, paraquat (which increases mitochondrial ROS production), and UV exposure (which generates large amounts of H_2O_2 within the cells). We also characterized the effect of resveratrol on vascular H_2O_2 production and antioxidant gene expression.

METHODS

Studies on endothelial cell cultures. Primary rat coronary arterial endothelial cells (Celprogen, San Pedro, CA) were maintained in

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culture as described (10, 15) in the presence or absence of resveratrol (10^{-6}–10^{-4} mol/l for 24 h). The cells were then treated with H_2O_2 (10^{-4}–10^{-3} mol/l) or paraquat (3 mmol/l) for 6 h (at 37°C) or exposed to UV light ($UV_{254\ nm}$, 100 J/m^2) and then incubated for 24 h in culture media. After the culture period, the cells were collected and frozen in liquid nitrogen.

In separate experiments, endothelial cells were treated with resveratrol (10^{-6}–10^{-4} mol/l for 24 h), followed by pretreatment with mercaptosuccinate (2 mmol/l, a glutathione peroxidase inhibitor, for 4 h) or 3-aminotriazole (25 μmol/l, a catalase inhbitor, for 4 h). Apopotosis was then induced by an administration of ox-LDL (40 μg/ml for 24 h; purchased from Biomedical Technologies, Stoughton, MA) or tumor necrosis factor-α (TNF-α, 10 ng/ml, for 24 h).

Vessel culture. To investigate the effects of resveratrol on endothelial cells in their native multicellular environment, aortic segments of rats were isolated and maintained in organoid culture under sterile conditions in F12 medium (GIBCO-BRL) containing antibiotics (100 UI/l penicillin, 100 mg/l streptomycin, and 10 μg/l Fungizone) and supplemented with 5% FCS (Boehringer-Mannheim) as previously described (15, 33, 49, 50, 52) for 18 h in the presence or absence of resveratrol (10^{-6}–10^{-4} mol/l). Vessel segments were then treated with H_2O_2 (10^{-4} and 10^{-3} mol/l) or paraquat (3 mmol/l) for 6 h (at 37°C) followed by washout and then incubated for 18 h. Other vessel segments were cut open, and the endothelial surface was exposed to UV light ($UV_{254\ nm}$, 100 J/m^2) and then incubated for 24 h in culture media. After the culture period, the vessels were frozen in liquid nitrogen.

In separate experiments, cultured arterial segments were treated with ox-LDL (40 μg/ml for 24 h) in the presence or absence of resveratrol (10^{-5} mol/l). To inhibit heme oxygenase-1 (HO-1), tin protoporphyrin IX (SnPPIX) (Porphyrin Products, Logan, UT) was added to the culture medium (50 μmol/l).

Caspase activity assay. The arterial and endothelial cell samples were homogenized in lyses buffer, and caspase activities were measured using Caspase-Glo 3/7 assay kit according to the manufacturer's instruction (Promega, Madison, WI). In 96-well plates, a 50-μl sample was mixed gently for 30 s with 50 μl Caspase-Glo 3/7 reagent and incubated for 2 h at room temperature. The lyses buffer with the reagent served as blank. Luminescence of the samples was measured using an Infinite M200 plate reader (Tecan, Research Triangle Park, NC). Luminescent intensity values were normalized to the sample protein concentration.

Detection of apoptotic cell death by ELISA. Endothelial cells exposed to oxidative stressors were lysed, and cytoplasmic histone-associated DNA fragments, which indicate apoptotic cell death, were quantified by the Cell Death Detection ELISA^{Plus} kit (Roche Diagnostics, Indianapolis, IN) as described (15). Results are reported as normalized arbitrary optical density units.

Measurement of H_2O_2 production. The cell-permeant oxidative fluorescent indicator dye 5(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate-acetyl ester (C-H_2DCFDA, Invitrogen, Carlsbad, CA) was used to assess H_2O_2 production in isolated arteries, as we reported (33). C-H_2DCFDA is a 2′7′-dichlorofluorescein derivative that has longer retention within the cells. In brief, vessel segments were treated with C-H_2DCFDA (10^{-5} mol/l at 37°C for 60 min). Untreated arteries were used as controls. The arteries were then washed three times. Fluorescent images of the endothelial layer of en face preparations were captured and analyzed using the Axiovision software (Carl Zeiss, Gottingen, Germany). Each experiment was performed in quadruplicates. Ten to fifteen entire fields per group were analyzed with one image per field. The background-corrected mean fluorescent intensities of each image were averaged. In some experiments, vessels coincubated with catalase were used as positive controls.

In separate experiments, dichlorofluorescein was incubated with H_2O_2 (10^{-3} mol/l for 20 min) in the presence or absence of resveratrol (10^{-6}–10^{-4} mol/l). Fluorescence of the samples was measured using an Infinite M200 plate reader (excitation, 485 nm; and emission, 535 nm; Tecan). In addition, the H_2O_2 scavenging effect of resveratrol was also assessed by using the homovanillic acid-horseradish peroxidase assay as reported (41). In brief, an assay mix consisting of homovanillic acid (100 μmol/l) and horseradish peroxidase (5 U/ml) in HEPES-buffered salt solution (pH 7.5) was incubated with H_2O_2 in the presence or absence of resveratrol (10^{-6}–10^{-4} mol/l) at 37°C. The reaction was stopped with 80 μl/ml glycine solution (0.1 mol/l, pH 10, 0°C). H_2O_2-induced fluorescent product was assessed by using an Infinite M200 fluorescent plate reader (excitation, 321 nm; and emission 421 nm). A calibration curve was constructed by using 0.01–100 μM H_2O_2 standards in assay mix (1 h at 37°C) with or without catalase (200 U/ml).

DNA damage analysis by comet assay. Endothelial cells were treated with resveratrol (10^{-5} mol/l for 24 h). After the culture period, the cells were harvested. Cells (10^5) in 100 μl PBS were mixed with 100 μl of 1.5% low-melting agarose, and 90 μl of this mixture were spotted on CometAssay slides (Trevigen, Gaithersburg, MD) between two layers of 1% low-melting agarose. DNA damage was induced by exposure of the slides to $UV_{254\ nm}$ (100 J/m^2). The extent of DNA fragmentation was examined by single-cell electrophoresis (comet assay), according to the modified protocols of Tice et al. (47). Briefly, the comet assay is based on the alkaline lysis of labile DNA at sites of damage. The slides were treated with a lysis solution (containing 2.5 M NaCl, 100 mM Na_2EDTA, 1% Triton X-100, 10% DMSO, and 10 mM Trizma base; pH 10, for 1 h at 4°C in the dark), rinsed with a neutralization buffer (3 × 5 min, 0.4 M Tris, pH 7.5) to remove detergents and salts, and then submerged in a high pH buffer (containing 300 mM NaOH and 1 mM Na_2EDTA, pH > 13) for 20 min to allow for unwinding of the DNA and the expression of alkali-labile damage. Electrophoresis was performed by using the same buffer at 25 V (~0.74 V/cm) and 300 mA for 20 min. At the end of the run, the slides were neutralized in 0.4 M Tris·HCl (pH 7.5), submersed in absolute ethanol for 3 min, and air dried, and DNA was stained with SYBR green (Invitrogen). Fluorescent images of the nuclei were captured by using a fluorescent microscope (at ×40 magnification). DNA damage was quantified by measuring the tail DNA content (as a percentage of total DNA) using the VisComet 2.0 software (Impulse).

Western blot analysis. To determine whether resveratrol alters the expression of major cellular antioxidant systems, Western blot analysis was performed as described (13, 14), using primary antibodies directed against catalase (no. 16731, Abcam, Cambridge, MA), glutathione peroxidase-1 (no. 16798, Abcam), Mn-SOD (no. SOD-110, Stressgen, Ann Arbor, MI), Cu,Zn-SOD (no. SOD-100, Stressgen), and HO-1 (no. AB1284, Chemicon/Millipore, Billerica, MA). Anti-β-actin (no. 6276, Abcam) was used for normalization for loading variability.

Data analysis. Data were normalized to the respective control mean values and are expressed as means ± SE. Statistical analyses of data were performed by Student's *t*-test or by two-way ANOVA followed by the Tukey post hoc test, as appropriate. $P < 0.05$ was considered statistically significant.

RESULTS

Resveratrol prevents oxidative stress-induced apoptotic cell death. Previously we have demonstrated that DNA fragmentation and caspase activities are good measures of endothelial apoptotic cell death in blood vessels (15, 33). In cultured endothelial cells, TNF-α (Fig. 1*A*) and ox-LDL (Fig. 1*B*) significantly increased caspase-3/7 activity. Pretreatment with resveratrol prevented both TNF-α- and ox-LDL-induced apoptotic cell death in a concentration-dependent manner (Fig. 1, *A* and *B*). In the resveratrol-treated cells, the glutathione peroxidase was inhibited by mercaptosuccinate and ox-LDL-induced

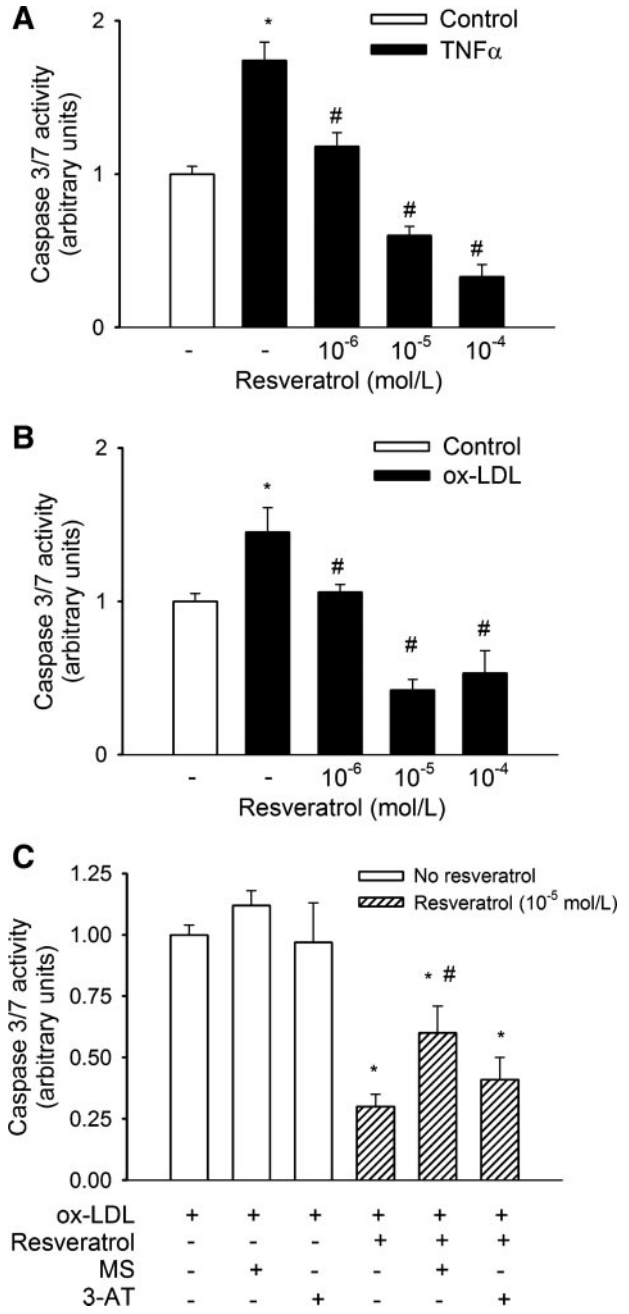

Fig. 1. Resveratrol pretreatment inhibits increases in caspase-3/7 activity in cultured endothelial cells induced by TNF-α (10 ng/ml; *A*) and oxidized LDL (ox-LDL, 40 μg/ml; *B*). Data are means ± SE; $n = 4–6$ experiments for each data point. *$P < 0.05$ vs. untreated control; #$P < 0.05$ vs. no resveratrol. *C*: effect of pretreatment with the glutathione peroxidase inhibitor mercaptosuccinate (MS, 2 mmol/l for 4 h) or the catalase inhibitor 3-aminotriazole (3-AT; 25 μmol/l for 4 h) on resveratrol (10^{-5} mol/l)-induced protection against ox-LDL-induced endothelial apoptosis. Data are means ± SE; $n = 4–6$ experiments for each data point. *$P < 0.05$ vs. ox-LDL only; #$P < 0.05$ vs. no MS or 3-AT.

endothelial apoptosis was increased (Fig. 1*C*). Administration of 3-aminotriazole also tended to increase ox-LDL-induced endothelial apoptosis; however, the difference did not reach statistical significance (Fig. 1*C*). Treatment of endothelial cells with H_2O_2 and paraquat or exposure to UV_{254} also significantly increased caspase-3/7 activity and DNA fragmentation, which were inhibited by pretreatment with resveratrol (Fig. 2, *A* and *B*). With the use of cultured rat arteries, similar findings were obtained: resveratrol in the micromolar range inhibited increases in caspase-3/7 activity induced by 10^{-4} mol/l H_2O_2 (Fig. 3*A*). At higher concentrations, resveratrol was also effective in preventing apoptosis induced by high doses of H_2O_2 (10^{-3} mol/l), paraquat, and UV exposure (Fig. 3, *B–D*).

Resveratrol decreases H_2O_2 levels. In a cell-free assay, resveratrol effectively attenuated H_2O_2 (10^{-3} mol/l for 20 min)-induced increases in dichlorofluorescein fluorescence (Fig. 4*A*). Resveratrol also eliminated H_2O_2-induced increases in fluorescent product in a homovanillic acid-horseradish peroxidase assay, which is highly specific to H_2O_2 (Fig. 4*B*). In cultured arteries, both acute (1 h; Fig. 4*C*) and chronic (24 h; Fig. 4*D*) treatment with resveratrol elicited significant, dose-dependent decreases in H_2O_2 generation induced by paraquat and UV exposure.

Resveratrol protects endothelial cells against DNA damage. A random sample of 120 cells was analyzed from each slide. Figure 5*A* shows examples of images of the comet assay. In cells without UV_{254} treatment, all the DNA was confined to the nuclei, as indicated by the percentage of DNA in the tail (<5%). In cells with UV_{254}-induced DNA strand breaks, a fluorescent tail along the electric field was observed because small DNA fragments migrated out of the nuclei (Fig. 5, *A* and *B*; median value of DNA in the tail, 34%; $P < 0.01$ vs. untreated control). Pretreatment with resveratrol led to a significant decrease in DNA damage (Fig. 5*B*; median value of DNA in the tail, 23%; $P < 0.05$ vs. UV_{254} treatment alone).

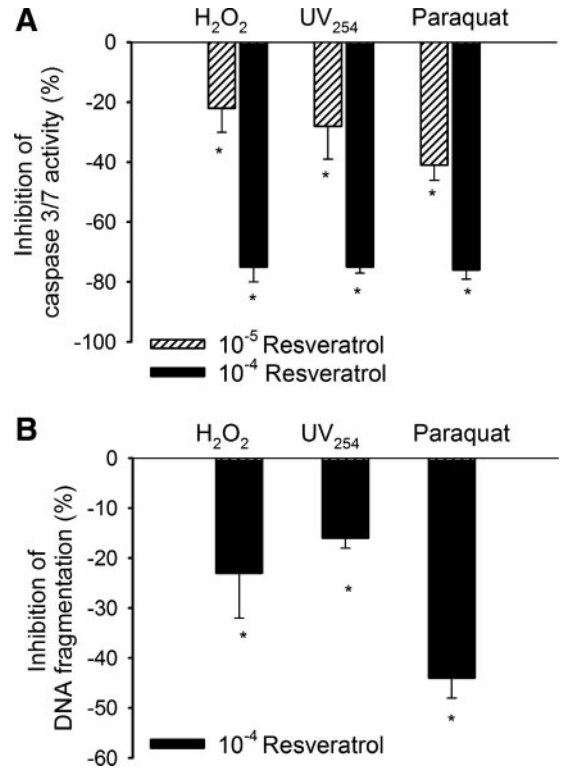

Fig. 2. Resveratrol pretreatment inhibits oxidative stressor (10^{-3} mol/l H_2O_2, 100 J/m^2 UV_{254}, or 3 mmol/l paraquat)-induced increases in caspase-3/7 activity (*A*) and DNA fragmentation (*B*) in cultured endothelial cells. Data are means ± SE; $n = 4–6$ experiments for each data point. *$P < 0.05$ vs. untreated control.

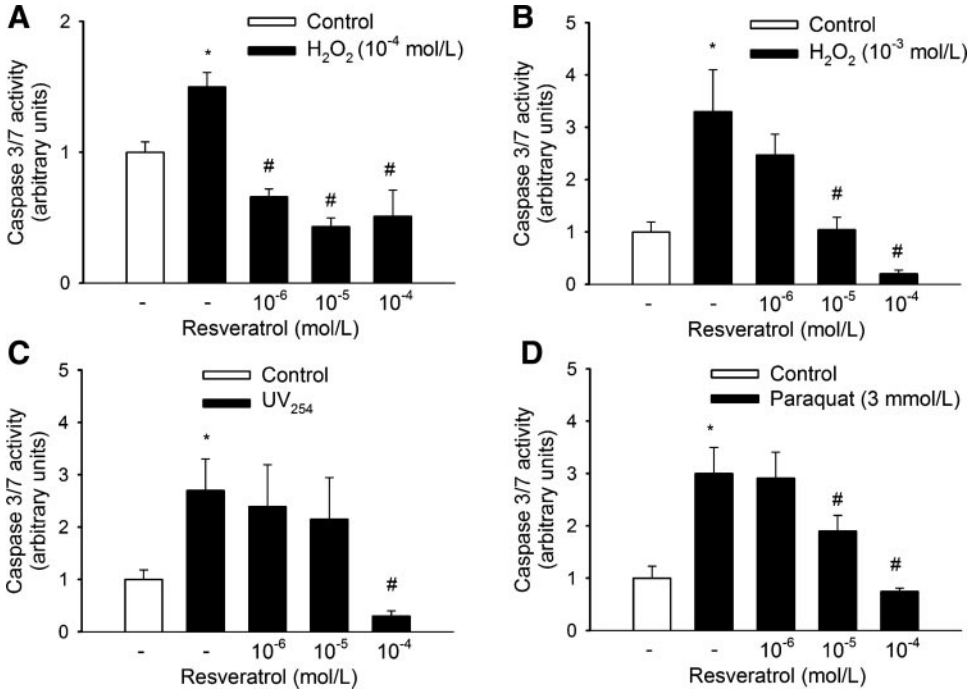

Fig. 3. Caspase-3/7 activity (see METHODS) in cultured rat aortic segments under control conditions and after H_2O_2 (10^{-4} and 10^{-3} mol/l; *A* and *B*, respectively) treatment or UV exposure (100 J/cm^2; *C*) or paraquat treatment (3 mmol/l; *D*). Effects of pretreatment with increasing concentrations of resveratrol are shown. Data are means ± SE; $n = 4–6$ experiments for each data point. *$P < 0.05$ vs. untreated control; #$P < 0.05$ vs. no resveratrol treatment.

Resveratrol upregulates antioxidant enzymes. Western blot analysis was performed to analyze resveratrol-induced changes in the vascular expression of antioxidant enzymes. Representative blots are shown in Fig. 6*A*, whereas Fig. 5, *B–E*, is summary data of densitometric analysis of the blots. Resveratrol treatment of cultured arteries had no significant effect on the expression of Cu,Zn-SOD (Fig. 6*B*) and Mn-SOD (Fig. 6*C*). In contrast, in a concentration-dependent manner, resveratrol upregulated catalase (Fig. 6, *A* and *D*) and glutathione peroxidase (Fig. 6, *A* and *E*).

Role of upregulation of HO-1 in the vasoprotective effects of resveratrol. Resveratrol treatment of cultured arteries significantly upregulated the expression of HO-1 (Fig. 7*A*). To determine whether HO-1 contributes to the vasoprotective

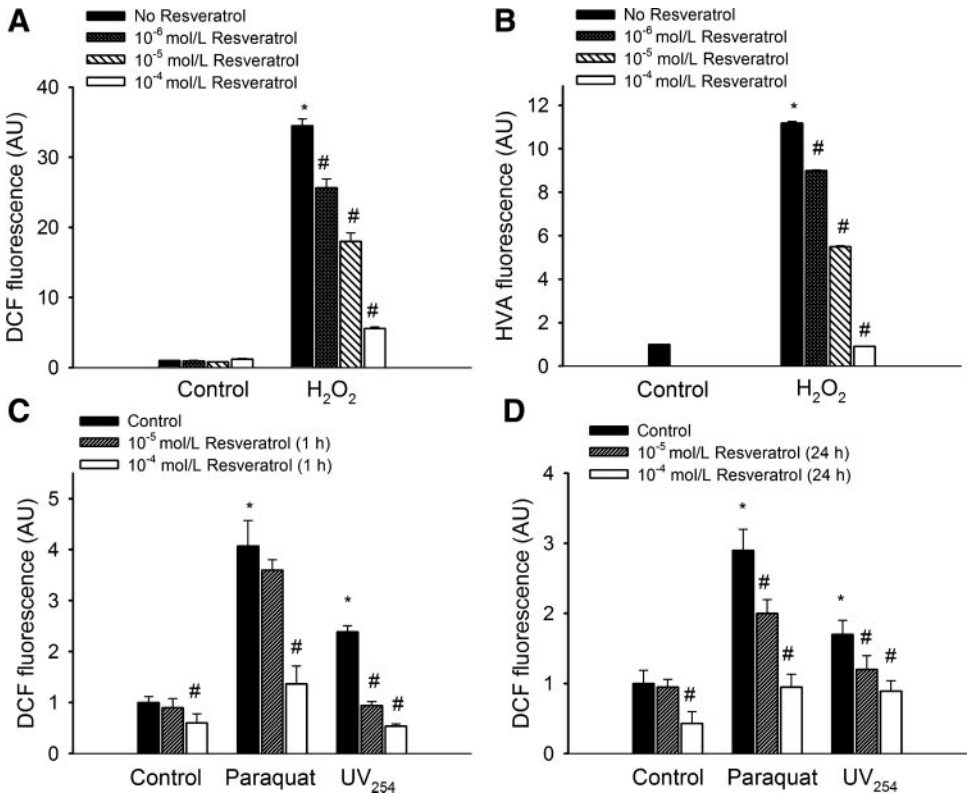

Fig. 4. *A*: resveratrol prevents H_2O_2-induced increases in 5(and 6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate-acetyl ester (DCF) fluorescence in cell-free solution. *B*: resveratrol prevents increases in fluorescent signal in a cell-free homovanillic acid (HVA)-horseradish peroxidase assay. *C* and *D*: both acute (1 h; *C*) and chronic (24 h; *D*) pretreatment with resveratrol inhibits H_2O_2 production (measured by the DCF fluorescence method) in endothelial cells of en face preparations of paraquat- and UV_{254}-exposed cultured rat aortic segments. AU, arbitrary units. $P < 0.05$ vs. untreated control; #$P < 0.05$ vs. no resveratrol treatment.

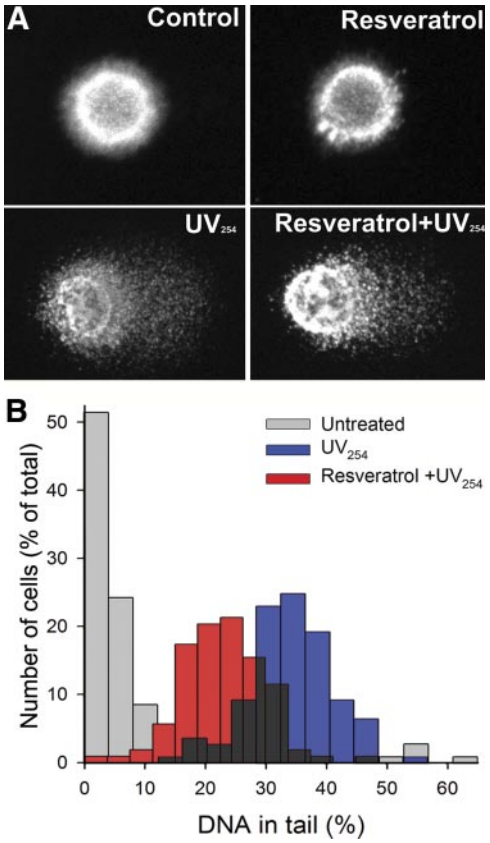

Fig. 5. *A*: representative comet assay images of endothelial cell nuclei. Endothelial cells were treated with resveratrol (10^{-5} mol/l for 24 h) followed by exposure to $UV_{254\ nm}$ (100 J/m^2). Damaged DNA migrates during electrophoresis from the nucleus toward the anode, forming a shape of a comet with a head (cell nucleus with intact DNA) and a tail (relaxed and broken DNA). Original magnification, ×20. *B*: frequency distribution of tail DNA content in untreated control and $UV_{254\ nm}$-exposed endothelial cells. Resveratrol pretreatment significantly attenuated $UV_{254\ nm}$-induced increases in tail DNA content.

effects of resveratrol, we inhibited HO-1 with SnPPIX. Our findings that, in control vessels, resveratrol inhibited ox-LDL-induced increases in vascular caspase-3/7 activity (Fig. 7*B*) are in accord with our observations in cultured endothelial cells (Fig. 1). In the presence of SnPPIX, ox-LDL-induced vascular caspase-3/7 activity was significantly higher (Fig. 7*B*), suggesting that resveratrol did not prevent ox-LDL-induced apoptosis if HO-1 was inhibited.

DISCUSSION

There are two major findings in this study: in blood vessels, resveratrol *1*) attenuates oxidative stress-induced cell death and *2*) exerts antioxidant effects, in part, by upregulating vascular antioxidant systems.

First, we have shown that TNF-α and ox-LDL induce apoptosis in endothelial cells, which was prevented by resvera-

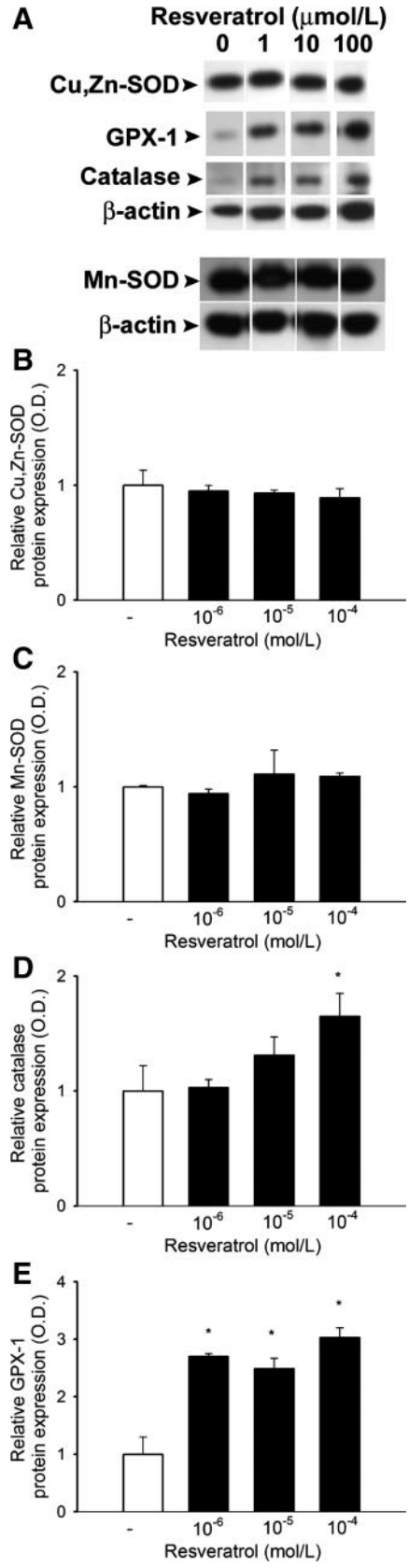

Fig. 6. *A*: representative bands from Western blots showing expression of Cu,Zn-SOD, Mn-SOD, catalase, and glutathione peroxidase (GPX-1) in rat aortic segments incubated (for 24 h) with increasing concentrations of resveratrol. Please note that β-actin content was used as loading control. Four samples per group were loaded in each gel, and each experiment was performed in duplicates with identical results. *B–E*: densitometric data (means ± SE). OD, optical density. *$P < 0.05$ vs. untreated.

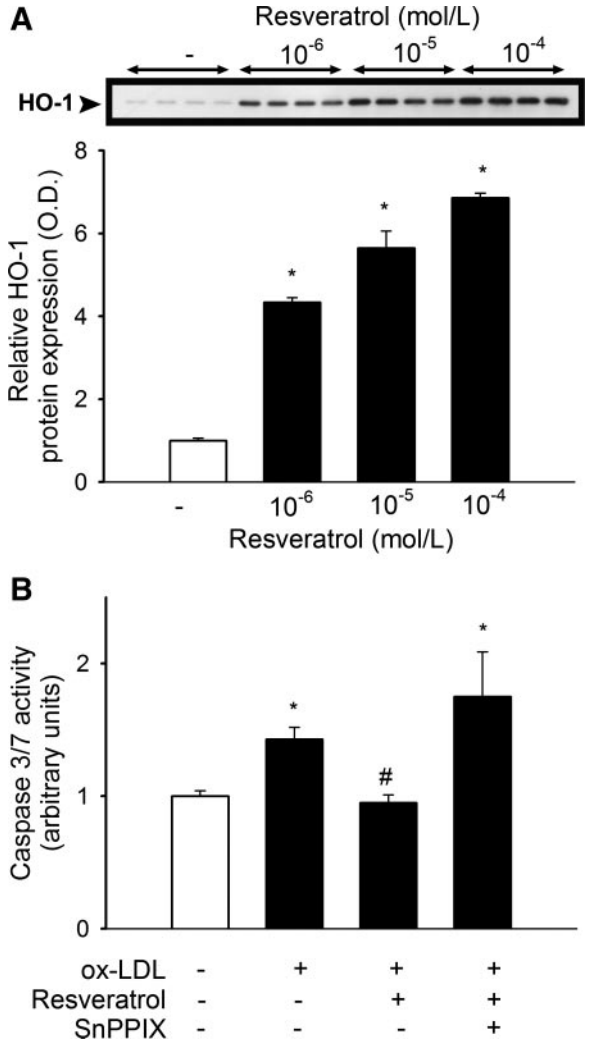

Fig. 7. *A*: original Western blot showing expression of heme oxygenase-1 (HO-1) in rat arterial segments incubated (for 24 h) with increasing concentrations of resveratrol. Bar graphs are densitometric data (means ± SE). *B*: in cultured arteries, resveratrol (10^{-5} mol/l) inhibited ox-LDL-induced increases in caspase-3/7 activity. Inhibition of HO-1 with tn protoporphyrin-IX (SnPPIX) antagonized the antiapoptotic effects of resveratrol. Data are means ± SE. *$P < 0.05$ vs. untreated; #$P < 0.05$ vs. no resveratrol.

trol in a dose-dependent manner (Fig. 1, *A* and *B*), extending recent findings (21, 27, 37). There is accumulating evidence that increased endothelial cell apoptosis may initiate atherosclerosis, whereas, at later phases of atherogenesis, an increased rate of apoptosis of endothelial cells, vascular smooth muscle cells, and macrophages were observed in vulnerable lesions and at sites of plaque rupture (reviewed in Ref. 9). Because both TNF-α and ox-LDL are physiologically relevant stimuli of apoptosis and both play an important role in the development of coronary artery disease, it is likely that antiapoptotic action of resveratrol will contribute to its cardioprotective effects in vivo. It is possible that antiapoptotic effects of resveratrol would promote the regression and/or stabilization of the atherosclerotic plaques in cardiac patients. Recent studies demonstrating that in apolipoprotein-E knockout mice resveratrol treatment significantly reduced the presence of atherosclerotic plaques in the aorta support this premise (40). Because both TNF-α and ox-LDL elicit endothelial oxidative stress in the coronary arterial endothelium (44, 49) and the inhibition of glutathione peroxidase significantly attenuated the antiapoptotic effect of resveratrol (Fig. 1*C*), we hypothesized that resveratrol may interfere with the H_2O_2-mediated proapoptotic mechanism.

To test this hypothesis, we have investigated endothelial apoptosis induced by exogenous H_2O_2, UV_{254} exposure, and paraquat. Although these stimuli are not physiological and do not play a role in atherogenesis, they are well-characterized oxidative stressors that are frequently used to assess oxidative stress resistance in cultured cells (33, 45). We found that exogenous H_2O_2 induced apoptosis both in cultured endothelial cells and cultured blood vessels, which was prevented by resveratrol in a dose-dependent manner (Figs. 2 and 3, *A* and *B*). Resveratrol also exerted protective effects against apoptotic cell death induced by intracellular H_2O_2 generation (UV treatment; Figs. 2 and 3*C*) or mitochondrial oxidative stress (paraquat; Figs. 2 and 3*D*).

The concentrations of resveratrol required to suppress oxidative stress-induced endothelial apoptosis in the present study overlapped those reported to inhibit endothelial activation, monocyte adhesion, NF-κB activation, platelet aggregation, and LDL oxidation and to regulate smooth muscle cell proliferation (12, 35, 46, 54). Our recent and previous (12, 35) results and findings by other laboratories (21, 38) indicate that micromolar levels of resveratrol are sufficient to exert vasculoprotective effects and attenuate vascular proinflammatory phenotypic alterations. With the consideration that each gram of fresh grape skin contains 50–100 μg resveratrol and red wines have 1.5–3 mg/l, the resveratrol concentrations used in these studies are achievable in vivo by the consumption of grapes, berries, red wine, and/or dietary supplements containing resveratrol (for an excellent review on the potential of resveratrol as a therapeutic agent for humans, see Ref. 7). In addition, it is likely that derivatives of resveratrol that exhibit higher bioavailability, slower clearance, and superior accumulation in tissues will be soon available for pharmacological vasculoprotection. Finally, it is also important to note that many studies showed that other phenols which are likely to exert resveratrol-like biological actions are also present in high concentrations in the Mediterranean diet and in red wines (5, 43, 59).

The mechanisms of antiapoptotic and vasculoprotective action of resveratrol are likely multifaceted (35). Our data showing that resveratrol in vitro effectively lowers H_2O_2 levels (Fig. 4, *A* and *B*) support the view that resveratrol itself exerts antioxidant effects likely due to the redox properties of the phenolic hydroxyl groups in its structure (36). Direct scavenging of H_2O_2 may explain the findings that paraquat- and UV treatment-induced increases in endothelial H_2O_2 generation were significantly attenuated by both acute and chronic pretreatment with resveratrol (Fig. 4, *C* and *D*). Pretreatment of endothelial cells with resveratrol also attenuated UV treatment-induced DNA damage (Fig. 5), which might reflect its ability to protect against increased cellular production of H_2O_2. It has been proposed that antioxidant activity of resveratrol may also reduce LDL oxidation (22).

In addition to the direct antioxidant effect of resveratrol, its chronic presence also upregulates the vascular expression of enzymes involved in scavenging of H_2O_2 (42), such as glutathione peroxidase (Fig. 6). Our findings accord with the results

of previous studies showing that in vitro treatment with resveratrol results in significant increases in H_2O_2 scavenging capacity in cardiomyocytes (8). The functional relevance of upregulation of glutathione peroxidase is supported by the finding that inhibition of glutathione peroxidase significantly attenuated the antiapoptotic effect of resveratrol (Fig. 1*C*). Interestingly, upregulation of glutathione peroxidase was observed even at lower resveratrol concentrations, whereas higher resveratrol doses were needed to induce catalase (Fig. 6). This observation may explain the finding that inhibition of catalase exerted less pronounced effects on resveratrol-induced cytoprotection (Fig. 1*C*).

We also found that resveratrol induces vascular HO-1 expression (Fig. 7*A*), and inhibition of HO-1 attenuates the antiapoptotic effects of resveratrol (Fig. 7*B*). It is significant that recently HO-1 induction was also shown to play a central role in resveratrol preconditioning of the heart (16). Upregulation of HO-1 was also shown to exert an antiapoptotic effect in the cardiovascular system in pathophysiological conditions associated with an increased oxidative stress, such as diabetes and ischemia-reperfusion (1, 2, 31). Further studies are definitely needed to characterize the effect of resveratrol on HO-1 in endothelial cells, including the signaling mechanisms responsible for the upregulation of HO-1 and its role in the regulation of the vascular redox state (48).

In addition to the prevention of oxidative stress-induced apoptotic cell death, the dual H_2O_2-lowering effects of resveratrol (both direct scavenging of H_2O_2 and upregulation of antioxidant enzymes) are likely to contribute to its inhibitory effect on H_2O_2-induced endothelial activation and NF-κB induction (12, 35). At present, the mechanisms by which resveratrol regulates gene expression are less understood. Resveratrol is a putative activator of sirtuin 1 (SIRT1) (28, 58), a NAD-dependent histone deacetylase, which may act as a survival factor (53) and is involved in the transcriptional regulation of a number of genes. Recent studies have demonstrated that SIRT1 is an important regulator of oxidative stress-induced mesangial cell apoptosis (32). Whether SIRT1 plays a role in the vasculoprotective effects of resveratrol is yet to be determined.

In a series of landmark studies, Dr. David Sinclair's group has demonstrated that resveratrol extends life span in lower organisms (28, 58), and there is good reason to believe that it also exerts antiaging activity in mammalian cells (6) (recently reviewed in Ref. 35). Vascular aging is characterized by increased oxidative stress (11, 13, 35), associated with an enhanced rate of endothelial apoptosis (15) and vascular inflammation (51). Thus future studies should elucidate whether antioxidant, antiapoptotic, and anti-inflammatory actions of resveratrol contribute to its antiaging vasculoprotective effects in the elderly (35).

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