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Resveratrol scavenges reactive oxygen species and effects radical-induced cellular responses

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Abstract

Scavenging or quenching of the reactive oxygen species (ROS) involved in oxidative stress has been the subject of many recent studies. Resveratrol, found in various natural food products, has been linked to decreased coronary artery disease and preventing cancer development. The present study measured the effect of resveratrol on several different systems involving the hydroxyl, superoxide, metal/enzymatic-induced, and cellular generated radicals. The rate constant for reaction of resveratrol with the hydroxyl radical was determined, and resveratrol was found to be an effective scavenger of hydroxyl, superoxide, and metal-induced radicals as well as showing antioxidant abilities in cells producing ROS. Resveratrol exhibits a protective effect against lipid peroxidation in cell membranes and DNA damage caused by ROS. Resveratrol was also found to have a significant inhibitory effect on the NF-κB signaling pathway after cellular exposure to metal-induced radicals. It was concluded that resveratrol in foods plays an important antioxidant role.

Keywords: Resveratrol; Hydroxyl radicals; Superoxide radicals; Cr(VI); DNA damage; Lipid peroxidation; NF-κB activation

Resveratrol has been cited in recent investigations for its possible antioxidant role and protective effects against certain forms of oxidant damage. Resveratrol is found in grapes, red wine, mulberries, and other food products. The antioxidant capacities of a wide variety of fruits have been reported by other laboratories [1–3]. Resveratrol is considered to be one of the major constituent antioxidants in red wine [4], which contains 6.5 mg/L resveratrol [5,6]. At present, it is difficult to calculate the amount of resveratrol which can be distributed to the organs and tissues of an animal after digestion. Antioxidants are found at higher concentrations in red wine than in white wine [7–10]. In addition to resveratrol, myricetin, quercetin, kaempferol, (−)-epicatechin-gallic acid, and (+)-catechin are also present in red wine [11,12]. Intake of moderate levels of red wine has been shown to decrease mortality associated with coronary artery disease [13]. Results of other studies have also demonstrated the ability of resveratrol to act as a powerful anti-inflammatory agent and to detoxify superoxide [14–18]. Resveratrol has recently been shown to function as a cancer chemoprevention agent [19], an anti-mutagenic agent, and an anti-initiative agent [20]. Resveratrol has also been shown to inhibit the enzymatic activity of the Cox-1 and -2, which are related to inflammation, in cell free models [21–23]. Additionally, PKC- and AP-1-mediated gene expression has been found to be suppressed by resveratrol in mammary epithelial cells [24]. Furthermore, some phenolic compounds, including resveratrol, were reported to inhibit TPA or UV-induced AP-1-mediated activity [25]. The phenolic structure of resveratrol is shown in Fig. 1.

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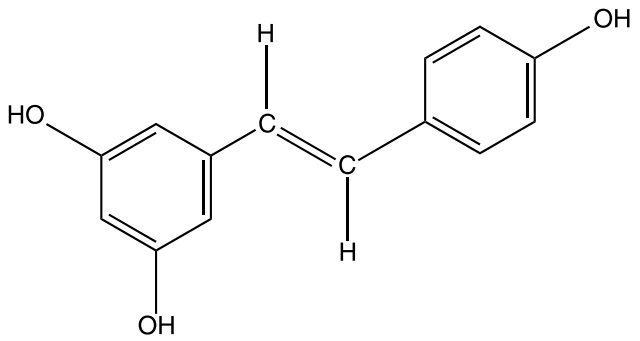

Fig. 1. The chemical structure of (trans-3,4′,-5-trihydroxystilebene) resveratrol.

Reactive oxygen species (ROS) include, but are not limited to, hydroxyl radical (·OH), superoxide radical ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2). ROS-mediated reactions have been shown to be involved in various pathogenic processes [26–28]. Radicals can be involved as initiators of the oxidative process [29] as well as playing an important role in the development of certain diseases [30,31]. Organisms produce antioxidants, such as catalase, superoxide dismutase, and uric acid, as part of a defense system against ROS-mediated cellular injury. When the defense system is jeopardized due to excessive generation of ROS, oxidative stress, or redox imbalance, may take place. This can cause damage to the organism [32,33], resulting in disease initiation [34]. ROS have also been shown to play an important role in carcinogenesis by damaging DNA and acting as tumor promoters [26,27].

Although recent studies have suggested that resveratrol functions as a scavenger of ROS and other free radicals, its antioxidant properties have not been well defined. The present study seeks to determine the reaction rate constant of resveratrol with ·OH, one of the most reactive free radicals. The determination of the rate constant is important in comparing its antioxidant properties to those of other well-established antioxidants. The present study uses the Fenton reaction as a source of ·OH to measure resveratrol's reaction rate with ·OH and its ability to scavenge the ·OH radical. Scavenging of the $O_2^{\cdot-}$ radical by resveratrol was performed using the reaction between xanthine and xanthine oxidase as a source of $O_2^{\cdot-}$ radicals. Radicals generated by metal and enzymatic systems were investigated by the use of the Cr(VI), NADPH/GSSG-R system. Cr(VI) was also used to determine the ability of resveratrol to scavenge radicals generated by cells exposed to this metal. The present study uses the Fenton reaction to generate ·OH radicals and measures the ability of resveratrol to inhibit damage to cell membranes in this system. Cell membrane damage is a key factor of free radical damage to organisms. The effect of resveratrol on DNA damage was performed by using the Fenton reaction and λ*Hin*dIII DNA fragments. Cr(VI)-induced NF-κB activation was used as a model system to examine the possible protective effects of resveratrol. The major questions to be addressed by the present study are: (1) What is the reaction rate constant between resveratrol and the ·OH radical? (2) Does resveratrol scavenge the $O_2^{\cdot-}$ radical? (3) Can resveratrol scavenge radicals from a metal-induced system? (4) Is resveratrol able to scavenge radicals which are generated from cells? (5) Does resveratrol inhibit lipid peroxidation caused by radicals? (6) Can resveratrol protect DNA from damage caused by ·OH radicals? (7) Does resveratrol inhibit Cr(VI)-induced activation of the NF-κB transcription factor?

Materials and methods

Materials. Resveratrol, glutathione reductase (GSSG-R), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), $FeSO_4$, H_2O_2, xanthine, xanthine oxidase, sodium formate, sodium dichromate ($Na_2Cr_2O_7$), Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin were purchased from Sigma (St. Louis, MO). 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from Aldrich (Milwaukee, WI). Phosphate-buffered saline (PBS) was purchased from Gibco-BRL (Gaithersburg, MD). Chelex 100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA). The RAW 264.7 cell line was purchased from American type culture collection (Rockville, MD). JB6 cells were kindly provided by Dr. Min Ding's laboratory. The spin trap, DMPO, was purified by charcoal decolorization and vacuum distillation. The DMPO solution, thus purified, did not contain any electron spin resonance (ESR) detectable impurities. The phosphate buffer (pH 7.4) was treated with Chelex 100 to remove transition metal ion contaminants.

Cell culture. RAW 264.7 cells were cultured in DMEM with 10% FBS, 2 mM L-glutamine, and 50 mg/ml pen/strep at 37 °C in a 5% CO_2 incubator. JB6 cells were cultured in DMEM with 1% FBS, 2 mM L-glutamine, and 50 mg/ml pen/strep at 37 °C in a 5% CO_2 incubator. Cells used for the luciferase assay were stably transfected with the NF-κB luciferase reporter plasmid [35] and then cultured under the same conditions as above.

Free radical measurements. ESR spin trapping was used to detect short-lived free radical intermediates. This technique involves the addition-type reaction of a short-lived radical with a paramagnetic compound (spin trap) to form a relatively long-lived free radical product (spin adduct), which can then be studied using conventional ESR. The intensity of the signal is used to measure the amount of short-lived radicals trapped, and the hyperfine couplings of the spin adduct are generally characteristic of the original trapped radicals. The spin trapping is the method of choice for detection and identification of free radical generation due to its specificity and sensitivity. All ESR measurements were conducted using a Bruker EMX spectrometer (Bruker Instruments, Billerica, MA) and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using potassium tetraperoxochromate (K_3CrO_8) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reference standards [36]. The relative radical concentration was estimated by multiplying half of the peak height by $(\Delta H_{pp})^2$, where ΔH_{pp} represents the peak-to-peak width. The Acquisit program was used for data acquisitions and analyses.

Reactants were mixed in test tubes in a final volume of 1.0 ml. The reaction mixture was then transferred to a flat cell for ESR measurement. The concentrations given in the figure legends are final concentrations. Experiments were performed at room temperature and under ambient air.

Lipid peroxidation. Lipid peroxidation of RAW 264.7 mouse peritoneal monocytes was measured by using a colorimetric assay for lipid peroxidation (LPO-586 Oxis International, Portland, OR). A typical reaction mixture contained $FeSO_4$ (0.1 mM), H_2O_2 (1 mM), and 1×10^7 cells in a total volume of 1.0 ml PBS (pH 7.4). Resveratrol was added to this mixture in concentrations ranging from 0.1 to 0.025 mM to measure its effect on lipid peroxidation. The mixture was exposed for 1 h in a shaking water bath at 37 °C. The measurement of lipid peroxidation is based on the reaction of a chromogenic reagent with malonaldehyde and 4-hydroxyalkenals at 45 °C. [37] The absorbance of the supernate was measured at 586 nm. The percentage of inhibition caused by resveratrol was calculated by comparing values to the control reaction.

DNA damage. The DNA strand break assay was carried out according to methods described earlier [38]. Briefly, reactions were performed in phosphate-buffered saline (pH 7.4) in 1.5 ml polypropylene tubes at 37 °C. Each reaction mixture contained 10 μg DNA (λ*Hin*dIII fragments), $FeSO_4$, H_2O_2, and various concentrations of resveratrol in a total volume of 100 μl buffer. To this solution, 2 μl of gel loading buffer (50 mM EDTA, 2.5% sodium dodecyl sulfate (SDS), and 0.1% bromophenol blue) was added and then electrophoresis was performed in 0.7% agarose at 1–2 V/cm in 40 mM Tris acetate buffer containing 2 mM EDTA (pH 8.0). Gels were stained in ethidium bromide (5 μg/ml) for 30 min and photographed under UV light using a Stratagene Eagle Eye II (Stratagene, La Jolla, CA).

Luciferase assay—assay of NF-κB activity in vitro. A confluent layer of JB6 cells transfected with NF-κB luciferase reporter was detached using trypsin and 5×10^4 viable cells, suspended in 1 ml DMEM supplemented with 1% fetal bovine serum (FBS), were added to each well of a 24-well plate. Plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2. Twenty-four hours later, cells were cultured in DMEM supplemented with 1.0% FBS for 24 h to minimize basal NF-κB activity and then exposed to $Na_2Cr_2O_7$ and various resveratrol concentrations in the same media to monitor the effects on NF-κB induction. The cells were extracted with 200 μl of 1× lysis buffer provided in the Luciferase Assay Kit (Promega, Madison, WI). Luciferase activity was measured using a Monolight luminometer, model 3010 (Analytical Luminescence Laboratory, San Diego, CA). The results were expressed as relative NF-κB activity compared with untreated controls [39].

Statistics. Data expressed as means ± SEM ($n = 3$) for each group. One-way ANOVA test was performed using SigmaStat statistical software (Jandel Scientific, San Rafael, CA) to compare the responses between treatments. Statistical significance was set at $p < 0.05$.

Results

Scavenging of ·OH by resveratrol

The Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {\cdot}OH + OH^-$) was used as a source of ·OH radicals. The results of scavenging ·OH radicals generated with this reaction by the addition of resveratrol are shown in Fig. 2. Fig. 2A shows a spectrum of an aqueous solution containing Fe^{2+}, H_2O_2, and the spin trap DMPO in a phosphate-buffered saline solution. The spectrum is a 1:2:2:1 quartet with splittings at $a_N = a_H = 14.9$ gauss (G). These splitting constants and the 1:2:2:1 quartet are indicative of the DMPO/·OH adduct [40,41]. The addition of resveratrol reduced the DMPO/·OH spectra in a concentration-dependent manner, demonstrating resveratrol's ability to scavenge the ·OH radical (Figs. 2B–D). Experiments were also carried out with sodium formate in a competition reaction to establish that resveratrol was acting as a scavenger and not an inhibitor of the Fenton reaction (data not shown).

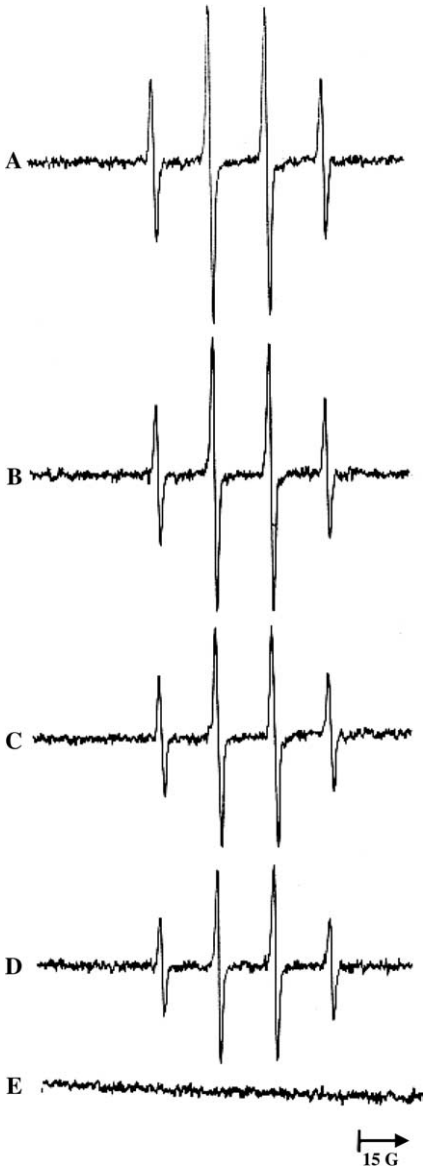

Fig. 2. ESR spectra recorded 3 min after reaction initiation from a phosphate-buffered solution (pH 7.4) containing 100 mM DMPO and the following reactants: (A) 1.0 mM $FeSO_4$ and 1.0 mM H_2O_2; (B) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2, and 0.650 mM resveratrol; (C) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2, and 0.975 mM resveratrol; (D) 1.0 mM $FeSO_4$, 1.0 mM H_2O_2, and 1.30 mM resveratrol; and (E) DMPO alone. The ESR spectrometer settings were: receiver gain, 2.52×10^5; time constant, 40 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3480 ± 100 G.

Calculation of reaction rate constant of resveratrol with ·OH

To determine the reaction rate of resveratrol with ·OH radicals, kinetic studies were conducted according

to methods outlined in our earlier studies [42,43]. The reaction steps can be written as:

$$\cdot OH + DMPO \xrightarrow{k_d} DMPO/\cdot OH \quad (1)$$

$$k_d = 2.1 \times 10^9\ M^{-1}\ s^{-1}$$

$$\cdot OH + Resveratrol \xrightarrow{k_R} product \quad (2)$$

$$-d[\cdot OH]/dt = k_d[DMPO][\cdot OH] + k_R[\cdot OH][Resveratrol] \quad (3)$$

$$d[DMPO/\cdot OH]/dt = k_d[DMPO][\cdot OH] \quad (4)$$

Dividing Eq. (3) by Eq. (4), one obtains

$$\frac{-d[\cdot OH]/dt}{d[DMPO/\cdot OH]/dt} = 1 + \frac{k_R[Resveratrol]}{k_d[DMPO]} \quad (5)$$

At a saturating level of DMPO and in the absence of resveratrol, the rates of ·OH spin trapping is equal to the rate of ·OH generation, $d[\cdot OH]/dt$. If V and v represent the rate of ·OH spin trapping in the absence and in the presence of resveratrol, respectively, one obtains

$$V/v = 1 + \frac{k_R[Resveratrol]}{k_d[DMPO]} \quad (6)$$

or

$$V/v - 1 = \frac{k_R[Resveratrol]}{k_d[DMPO]} \quad (7)$$

The rate curve shows the dose-dependent inhibition of ·OH by resveratrol. The data were plotted according to Eq. (7). A straight line was obtained with a slope of 0.45, which is the ratio of k_R/k_d. Using the value of $k_d = 2.1 \times 10^9\ M^{-1}\ s^{-1}$ for the ·OH trapping by DMPO, the value of k_R was calculated as follows:

$$k_R = 0.45(k_d)$$

$$= 0.45(2.1 \times 10^9\ M^{-1}\ s^{-1})$$

$$k_R = 9.45 \times 10^8\ M^{-1}\ s^{-1}$$

It should be noted that the rate constant k_R as calculated did not include the decay of the spin adduct after its formation. However, it does provide a convenient method for obtaining a relative value.

Scavenging of $O_2^{\cdot-}$ by resveratrol

Superoxide radicals were generated using a xanthine/xanthine oxidase system and measured using ESR. The spectrum in Fig. 3A shows the spin adduct spectrum generated from xanthine and xanthine oxidase in the presence of DMPO. Analysis of the spectrum shows hyperfine splittings at $a_N = 14.2$ G, $a_H = 11.5$ G, and $a_H^{\lambda} = 1.2$ G. These splittings are typical of the DMPO/$O_2^{\cdot-}$ spectra [42]. Figs. 3B–E show the reduction in signal effects of resveratrol on the DMPO/$O_2^{\cdot-}$ adduct signal in a concentration-dependent manner.

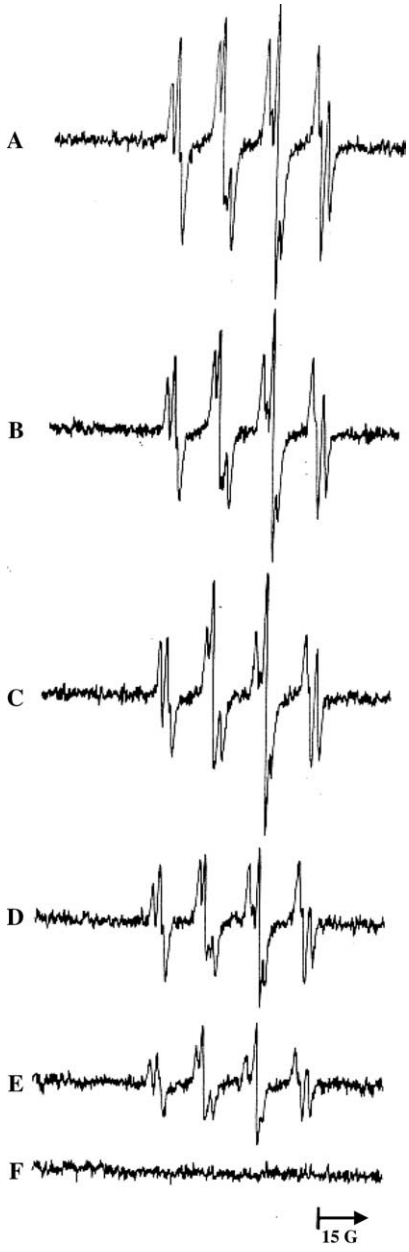

Fig. 3. ESR spectra recorded 1 min after reaction initiation from a phosphate-buffered solution (pH 7.4) containing 50 mM DMPO and the following reactants: (A) 3.5 mM xanthine and 2 U/ml xanthine oxidase; (B) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 0.325 mM resveratrol; (C) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 0.650 mM resveratrol; (D) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 0.975 mM resveratrol; (E) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 1.30 mM resveratrol; and (F) DMPO alone. The ESR spectrometer settings were: receiver gain, 2.52×10^4; time constant, 20 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3480 ± 100 G.

Effect on radicals induced by enzymatic reaction with Cr(VI)

Fig. 4A shows the spectrum obtained from the reaction between Cr(VI), NADPH, and GSSG-R in the presence of the spin trap DMPO. This spectrum consists

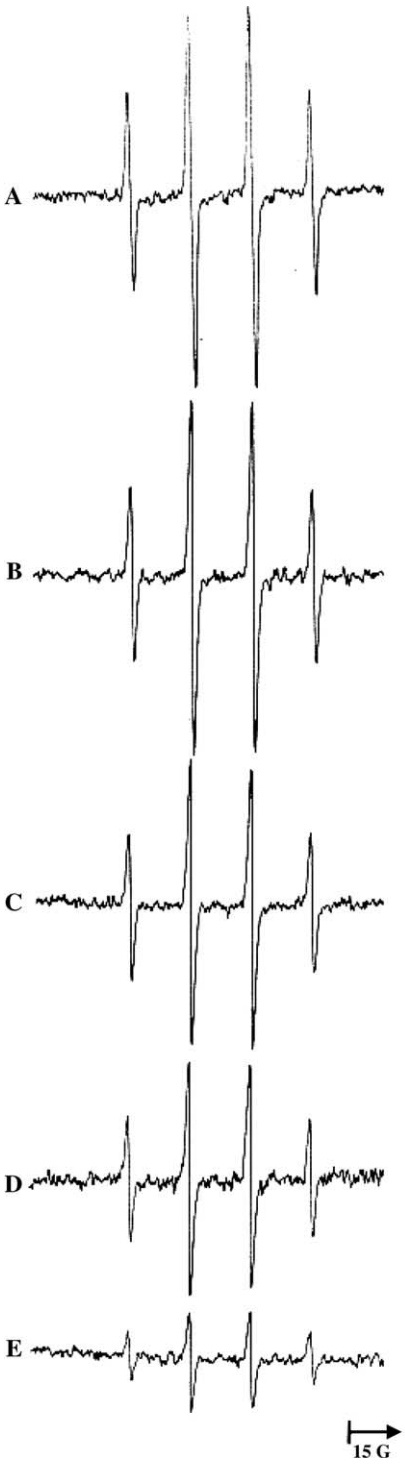

Fig. 4. ESR spectra recorded 3 min after reaction initiation, from a pH 7.4 phosphate-buffered solution containing 100 mM DMPO and the following reactants: (A) 2 mM Cr(VI), 1 mM NADPH, and 0.5 mg/ml GSSG-R; (B) 2 mM Cr(VI), 1 mM NADPH, 0.5 mg/ml GSSG-R, and 0.650 mM resveratrol; (C) 2 mM Cr(VI), 1 mM NADPH, 0.5 mg/ml GSSG-R, and 0.975 mM resveratrol; (D) 2 mM Cr(VI), 1 mM NADPH, 0.5 mg/ml GSSG-R, and 1.30 mM resveratrol; and (E) 2 mM Cr(VI), 1 mM NADPH, 0.5 mg/ml GSSG-R, and super saturated resveratrol. The ESR spectrometer settings were: receiver gain, 2.52×10^4; time constant, 40 ms; modulation amplitude, 0.98 G; scan time, 60 s; and magnetic field, 3490 ± 100 G.

of a 1:2:2:1 quartet with splittings at $a_H = a_N = 14.9$. Based on these splitting constants, the 1:2:2:1 quartet was assigned to the DMPO/·OH adduct [41,44–46]. Figs. 4B–D show the effects of resveratrol on the Cr(VI), NADPH/GSSG-R reaction and a decrease in the radical spin adduct, demonstrating reveratrol's ability to scavenge ·OH radicals.

Effect on radicals induced by cellular exposure to Cr(VI)

The generation of ·OH radical from Cr(VI)-stimulated RAW 264.7 cells in the presence of DMPO and the effects of resveratrol are displayed in Fig. 5. The spectrum in Fig. 5A shows the result of RAW 264.7 cells exposed to Cr(VI) in the presence of the spin trap DMPO. The spectrum exhibits the 1:2:2:1 quartet and hyperfine splitting associated with the DMPO/·OH adduct. Figs. 5B–D show the effect of adding increasing concentrations of resveratrol on the quantity of radicals generated by Cr(VI)-stimulated cells. A reduction in the amount of radicals trapped was observed, indicating that the ·OH radicals produced by these cells are scavenged by the resveratrol.

Inhibition of ·OH-induced lipid peroxidation

Lipid peroxidation is an indicator of possible free radical damage to cells. It has been demonstrated that ·OH radicals are able to cause cell membrane damage and initiate lipid peroxidation [47,48]. Fig. 6 displays the results of the measurement of lipid peroxidation by radicals generated in the Fenton reaction and the subsequent protective effects of resveratrol in RAW 264.7 cells. In the present study, measurements were made on lipid peroxidation in untreated control cells, cells exposed to the Fenton reaction, and cells exposed to the Fenton reaction with resveratrol treatment at various concentrations. The resveratrol protected the cells from the radicals generated by the Fenton reaction in a concentration-dependent manner.

Inhibition of hydroxyl radical ·OH-induced DNA damage

The Fenton reaction was used again to generate ·OH radicals. Resveratrol was observed to protect the DNA from the ·OH radical "fragmenting" effect on the λ*Hin*dIII DNA (Fig. 7). Lane 1 represents untreated λ*Hin*dIII DNA alone as a control. Lanes 7–9 show that DNA was damaged by the Fenton reaction when different amounts of H_2O_2 were present, resulting in smearing of the 7 DNA bands. Lanes 10–12 are identical to lanes 7–9 but with 0.5 mM resveratrol added. It should be noted that the smearing (lanes 10–12) was less noticeable and the DNA bands were more pronounced than in lanes 7–9. Lanes 2–4 show a higher concentration

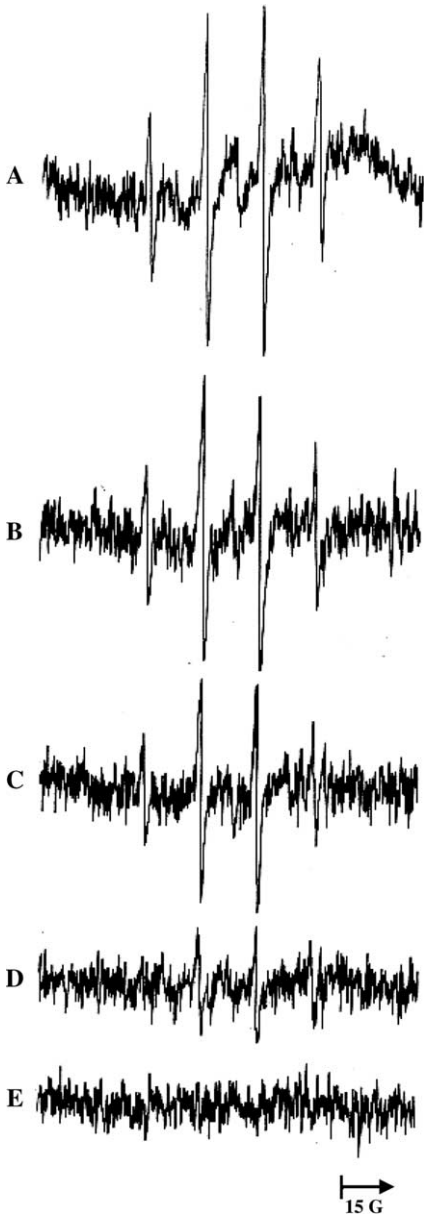

Fig. 5. ESR spectra recorded 5 min after the addition of Cr(VI) to cell suspension in a phosphate-buffered solution (pH 7.4) containing the following reactants: (A) 100 mM DMPO, 2 mM Cr(VI), and 1×10^6 RAW 264.7 cells; (B) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells, and 0.650 mM resveratrol; (C) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells, and 0.975 mM resveratrol; (D) 100 mM DMPO, 2 mM Cr(VI), 1×10^6 RAW 264.7 cells, and 1.30 mM resveratrol; and (E) 100 mM DMPO and 1×10^6 RAW 264.7 cells. The ESR spectrometer settings were: receiver gain, 5.02×10^4; time constant, 4 ms; modulation amplitude, 1.0 G; scan time, 60 s; and magnetic field, 3486 ± 100 G.

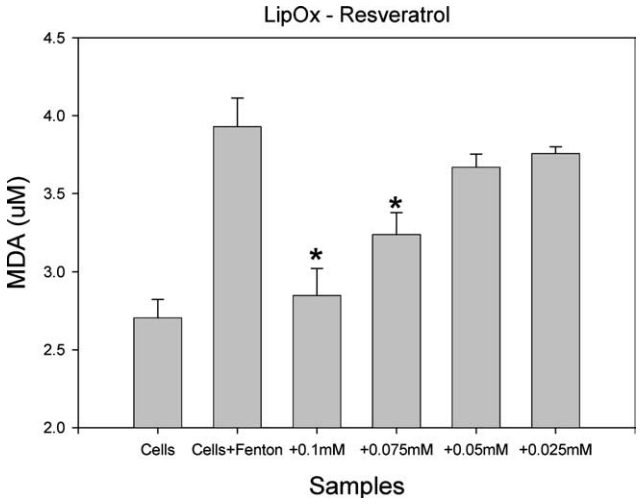

Fig. 6. Fenton reaction induced lipid peroxidation. Exposure mixture contained 1.0 mM $FeSO_4$, 0.1 mM H_2O_2, 5×10^7 RAW 264.7 cells, and between 0.025 mM and 0.1 mM concentrations of resveratrol. Data presented are means $\pm$ SD for 4 reps. of experiments. Asterisks indicate a significant decrease in lipid peroxidation compared to the positive control ($p < 0.05$).

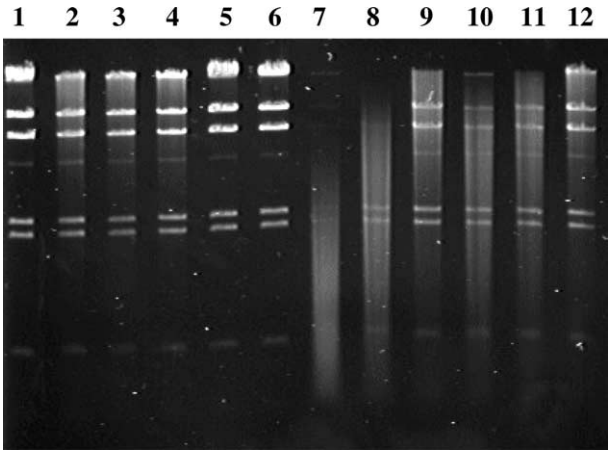

Fig. 7. DNA strand breaks in λ*Hin*dIII DNA induced by Fenton-mediated reactions. Lane 1, DNA alone; lane 2, DNA, 0.9 mM resveratrol, 1 mM $FeSO_4$, and 10 mM H_2O_2; lane 3, DNA, 0.9 mM resveratrol, 1 mM $FeSO_4$, and 5 mM H_2O_2; lane 4, DNA, 0.9 mM resveratrol, 1 mM $FeSO_4$, and 2 mM H_2O_2; lane 5, DNA and 0.9 mM resveratrol; lane 6, DNA and 0.5 mM resveratrol; lane 7, DNA, 1 mM $FeSO_4$, and 10 mM H_2O_2; lane 8, DNA, 1 mM $FeSO_4$, and 5 mM H_2O_2; lane 9, DNA, 1 mM $FeSO_4$, and 2 mM H_2O_2; lane 10, DNA, 0.5 mM resveratrol, 1 mM $FeSO_4$, and 10 mM H_2O_2; lane 11,DNA, 0.5 mM resveratrol, 1 mM $FeSO_4$, and 5 mM H_2O_2; and lane 12, DNA, 0.5 mM resveratrol, 1 mM $FeSO_4$, and 2 mM H_2O_2.

of resveratrol added (0.9 mM) to the Fenton reaction mixture and an even sharper DNA banding and less smearing compared to lanes 7–9. Lanes 5 and 6 show DNA exposed to resveratrol alone without the presence of Fenton reagents, DNA damage was not observed in this reaction condition.

Inhibition of Cr(VI)-induced NF-κB activation

The NF-κB activation in JB6 cells stimulated by exposure to different concentrations of Cr(VI) was measured using a luciferase reporter assay. Fig. 8 shows that Cr(VI) treatment of JB6 cells induced an increased

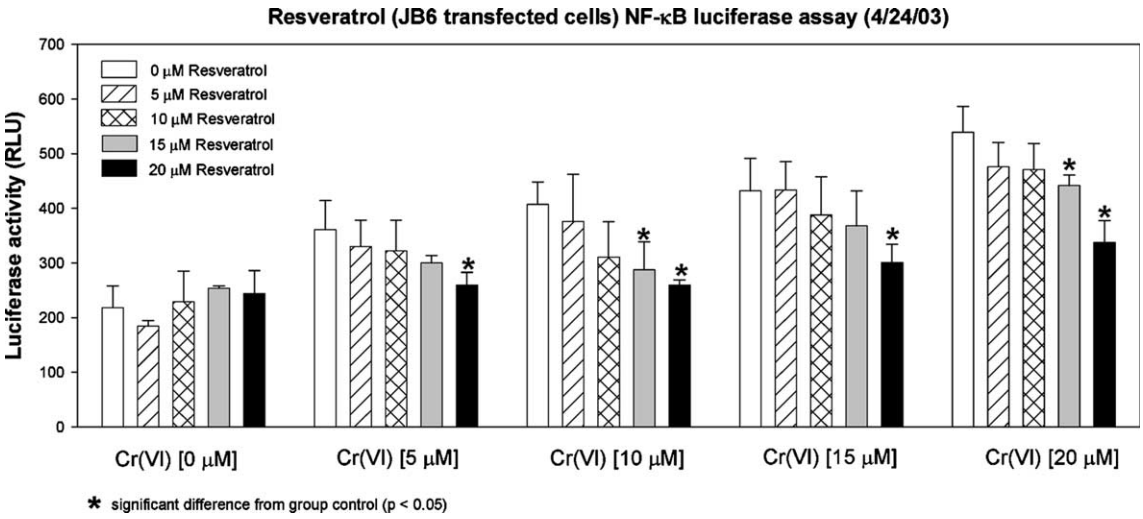

Fig. 8. Activation of NF-κB Cr(VI) in JB6 cells (5×10^4/ml), transfected with a NF-κB-dependent luciferase reporter plasmid. Cells were treated with Cr(VI), for an additional 12 h. The luciferase activity was determined as described in Materials and methods. The data presented are means ± SEM values of three independent experiments. * indicates a significant decrease from the positive control group ($p < 0.05$). RLU, relative light units.

luciferase gene activation relative to the control group. Increasing concentration of Cr(VI) caused a higher response from the luciferase reporter gene. This response was inhibited by the addition of resveratrol to the exposure group. At the high levels of resveratrol (15 and 20 μM) the inhibition was significant (Fig. 8).

Discussion

ROS and free radicals are involved in a variety of diseases and cellular response pathways. Organisms generate antioxidants, such as catalase, SOD, and uric acid, which act as endogenous antioxidant defenses. Recently, exogenous antioxidants introduced through diet or by other means are becoming popular. There has been a growing interest in the identification of possible dietary antioxidants to treat or prevent diseases caused by free radicals. These substances include fruits, vegetables, and teas, as well as their derivatives. Epidemiologic studies have shown the effectiveness of diets rich in fruits and vegetables in reducing the risks of cancer and other diseases [49]. ROS damage can be affected by two factors: (1) scavenging of radicals formed during reactions and (2) inhibiting the radical generation. The results of the present study indicate that resveratrol scavenged the radicals but did not inhibit their production as measured by spin trapping competitions using sodium formate as a second free radical scavenger (data not shown). Other investigators have indicated that resveratrol could be an effective antioxidant. For these reasons, we investigated the effect of resveratrol against specific radicals, its scavenging rate, and its specificity in protection against certain radical damage. The calculated resveratrol reaction rate with ·OH ($9.45 \times 10^8\ M^{-1}\ s^{-1}$) is significant but somewhat less than that of well-established antioxidants, such as ascorbate ($1.2 \times 10^{10}\ M^{-1}\ s^{-1}$), GSH ($1.5 \times 10^{10}\ M^{-1}\ s^{-1}$), and cysteine ($1.3 \times 10^{10}\ M^{-1}\ s^{-1}$). The antioxidant properties of resveratrol may be due to its phenolic groups (Fig. 1).

The Fenton reaction, involving Fe^{2+} and H_2O_2 for ·OH generation, was used to demonstrate resveratrol's ability to scavenge ·OH radicals and establish its concentration dependence. We also used the reaction between xanthine and xanthine oxidase to demonstrate the ability of resveratrol to scavenge $O_2^{\cdot-}$ radical and a Cr(VI) system to measure the effect of resveratrol in a transition metal-based radical generating system.

It should be noted that resveratrol was used in an aqueous medium in order to eliminate the scavenging interference presented by organic solvents, such as EtOH. This limited the concentrations which could be used to those of an aqueous environment. Using these concentrations, we were able to measure the rate constant, which was used to determine resveratrol's radical scavenging ability. Since DMPO is a radical scavenger and competes with resveratrol for radicals, a relatively high concentration of resveratrol must be used to measure its reaction constant with the ·OH radical.

Enzymatic reactions between Cr(VI), NADPH, and GSSG-R have been found to generate ·OH [50]. This same system has been used to show the ability of Cr(VI) to activate NF-κB, AP-1 [51,52], TNF-α [53], and other inflammatory indicators [54]. These species may be involved in the development of cancer resulting from Cr(VI) exposure [33]. The ability of resveratrol to scavenge and reduce radicals in this system supports resveratrol's possible anti-cancer ability. It also suggests that resveratrol

may affect other radical generating systems, such as nickel, silica, and asbestos, and reduce their carcinogenic potential.

The ability of resveratrol to scavenge radicals generated by cellular exposure to Cr(VI) further indicates resveratrol's radical scavenging ability in different types of ROS generation systems. RAW 264.7 activation by Cr(VI) has been used previously to show the oxidative stress that results from cellular stimulation by this transition metal [54]. Resveratrol affected this system at a non-lethal level (data not shown) and allowed evaluation of resveratrol under conditions more representative of in vivo exposures. Cellular systems generate a variety of radicals including peroxisome release of H_2O_2 and electron transport chain $O_2^{\cdot-}$ generation. The scavenging seen with ·OH and $O_2^{\cdot-}$ by resveratrol demonstrates its effectiveness against biologically generated radicals.

Another aspect of free radical damage involves injury to cellular membranes. Measurement of lipid peroxidation was used as an indicator of membrane damage in RAW 264.7 cells exposed to ·OH radicals generated from the Fenton reaction. Lipid peroxidation can cause a cascade effect of lipid-derived radicals, thereby causing additional membrane damage. The products of lipid peroxidation, malondialdehyde, and other groups of aldehyde products, such as hexanol, 4-hydroxylnonenal, and related aldehydes, may also cause DNA damage [55]. It has also been proposed that free radicals derived from lipid peroxidation may function as tumor initiators [56]. Our investigation shows that resveratrol was effective in inhibiting lipid peroxidation of cellular membranes.

The present investigation also examined the ability of resveratrol to inhibit DNA damage in λ*Hin*dIII DNA fragments from exposure to free radicals. The Fenton reaction between Fe^{2+} and H_2O_2 generates Fe^{3+}, OH^-, and ·OH [57]. The hydroxyl radicals produced can cause DNA strand breaks. This DNA damage by free radicals has been shown to play a key role in metal-induced carcinogenesis [58]. The results of the present study indicate that resveratrol can inhibit DNA damage caused by ·OH. Carcinogens, such as chromium, asbestos, silica, nickel, and possibly other metal systems, exert their carcinogenic effect, in part, through production of free radicals. The ability of resveratrol to scavenge free radicals demonstrates its possible preventative value in the inhibition of carcinogenesis involving free radical reactions.

Another important result of the present study was the indication that resveratrol can inhibit the activation of NF-κB caused by Cr(VI) exposure. NF-κB is involved in the inflammatory and carcinogenic signaling cascades, and its activation can be a marker of carcinogenesis [58]. The results demonstrating that resveratrol inhibits this important signaling factor, and its mechanism of inhibition may be due to its ability to scavenge several free radical species and, therefore, prevent the activation of NF-κB caused by free radicals.

In summary the results of the present investigation indicate the following: (1) Resveratrol is an efficient ·OH radical scavenger with a reaction rate constant of $k = 9.45 \times 10^8\ M^{-1}\ s^{-1}$, which is significant but somewhat less than recognized radical scavengers such as ascorbic acid and glutathione. (2) Resveratrol scavenged the $O_2^{\cdot-}$ radical. (3) Radicals produced by the enzymatic reaction with Cr(VI) were scavenged by resveratrol. (4) Resveratrol acts as a scavenger of radicals produced by cells after exposure to Cr(VI). (5) Lipid peroxidation in cell membranes caused by exposure to ·OH radicals was inhibited by resveratrol. (6) DNA damage due to ·OH radicals produced by the Fenton reaction was inhibited by resveratrol. (7) Finally, the NF-κB activation after cellular exposure to Cr(VI) was inhibited due to the presence of resveratrol.

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