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Resveratrol Is a Selective Human Cytochrome P450 1A1 Inhibitor

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Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is a phytoalexin compound found in juice and wine produced from dark-skinned grape cultivars and reported to have anti-inflammatory and anticarcinogenic activities. To investigate the mechanism of anticarcinogenic activities of resveratrol, the effects on cytochrome P450 (P450) were determined in human liver microsomes and *Escherichia coli* membranes coexpressing human P450 1A1 or P450 1A2 with human NADPH-P450 reductase (bicistronic expression system). Resveratrol slightly inhibited ethoxyresorufin *O*-deethylation (EROD) activity in human liver microsomes with an IC_{50} of 1.1 mM. Interestingly, resveratrol exhibited potent inhibition of human P450 1A1 in a dose-dependent manner with IC_{50} of 23 μM for EROD and IC_{50} of 11 μM for methoxyresorufin *O*-demethylation (MROD). However, the inhibition of human P450 1A2 by resveratrol was not so strong (IC_{50} 1.2 mM for EROD and 580 μM for MROD). Resveratrol showed over 50-fold selectivity for P450 1A1 over P450 1A2. The activities of human NADPH-P450 reductase were not significantly changed by resveratrol. In a human P450 1A1/reductase bicistronic expression system, resveratrol inhibited human P450 1A1 activity in a mixed-type inhibition (competitive-noncompetitive) with a K_i values of 9 and 89 μM. These results suggest that resveratrol is a selective human P450 1A1 inhibitor, and may be considered for use as a strong cancer chemopreventive agent in humans.

Resveratrol (3,4′,5-trihydroxystilbene; Fig. 1) occurs naturally in grapes and other plants in response to pathogen attack, UV irradiation, or exposure to ozone [1–3]. It is found in high concentrations in wine, particularly in red wine [4]. The protective effect of red wine toward coronary heart disease has been attributed to the antioxidant activity [5], modulation of the synthesis of hepatic apolipoprotein and lipids [6], inhibition of platelet aggregation [7, 8], and eiconanoid productions in human platelets and neutrophils [7, 9].

Resveratrol is one of the most promising cancer chemopreventive agents. It has been shown to inhibit various cellular events associated with carcinogenesis [10]. Resveratrol induced quinone reductase in cultured mouse hepatoma cells, inhibited cyclooxygenase and hydroperoxidase, and induced cell differentiation of HL-60 human promyelocytic leukemia cells. Moreover, it has shown to have an anti-tumorigenic effect in a two-stage mouse skin cancer model [10].

Human cytochrome P450 (P450) 1A1 is well known as a aryl hydrocarbon hydroxylase and involves in the activation of procarcinogens of the polycyclic aromatic hydrocarbons (PAHs). CYP1A1 is expressed at only very low levels in human liver and mainly expressed in human lung, placenta, and lymphocytes [11, 12]. This enzyme is often considered to be one of the most important enzymes involved in tumor initiation. Thus, potent inhibitors of P450 1A1 are good candidate chemopreventive agents for cancer, especially lung cancer.

In these studies, we have investigated the effect of resveratrol on the activities of human P450s 1A1 and 1A2 in order to elucidate the mechanism of anticarcinogenic effect, and we demonstrate that resveratrol is a selective P450 1A1 inhibitor in humans.

MATERIALS AND METHODS

Materials. Resveratrol, ethoxyresorufin, methoxyresorufin, resorufin, DMSO, thiamine, isopropyl-1-thio-β-D-galactopyranoside (IPTG), δ-aminolevulinic acid and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). Bactopeptone, yeast extract and bactoagar were obtained from Difco Lab. (Detroit, MI). Other chemicals were of highest grade commercially available.

Recombinant human P450s. Coexpression plasmids for human P450 (1A1 or 1A2) and NADPH-P450 reductase were transformed into *Escherichia coli* DH5α [13]. A single ampicillin-resistant colony of transformed cells was selected and grown in overnight culture to saturation at 37°C in LB medium containing 100 μg ampicillin ml^{-1}. A 10-ml aliquot was used to inoculate each liter of Terrific Broth (TB) containing 0.2% bactopeptone (w/v), 100 μg ampicillin ml^{-1}, 1.0 mM

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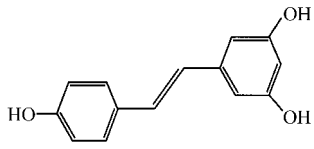

FIG. 1. Structure of resveratrol (*trans*-3,4′,5-trihydroxystilbene).

thiamine, trace elements, 0.5 mM δ-aminolevulinic acid, and 1.0 mM IPTG [14]. The cultures were grown at 30°C with shaking at 200 rpm for 24 h. Membrane fractions were prepared from bacteria as previously described [15].

Preparation of human liver microsomes. Frozen human liver samples were thawed in 0.1 M Tris acetate buffer (pH 7.4) containing 0.1 M KCl, 1.0 mM EDTA, and 20 μM butylated hydroxytoluene and homogenized in a Teflon-glass homogenizer. The homogenate was centrifuged at $10^4 \times g$ for 20 min at 4°C and the supernatant was centrifuged for 60 min at $10^5 \times g$ at 4°C. The microsomal pellet was resuspended in 10 mM Tris acetate buffer (pH 7.4) containing 1.0 mM EDTA and 20% glycerol (v/v). Protein concentrations were estimated using the bichinchoninic acid (BCA) method according to supplier's recommendations (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as a standard. The microsomes were stored at −80°C.

Enzyme assays. 7-Ethoxyresorufin *O*-deethylation (EROD) and 7-methoxyresorufin *O*-demethylation (MROD) activities were determined as previously described [16]. The reaction mixture contained 100 mM potassium phosphate buffer (pH 7.4), 2 mg BSA ml^{-1}, 10 μM dicoumarol, human liver microsomes or *E. coli* membranes, and 0.2 μM ethoxyresorufin or methoxyresorufin. The reaction mixtures were preincubated at 37°C for 3 min, and the reaction was initiated by addition of 120 μM NADPH. The formation of resorufin was determined fluorometrically with a Perkin-Elmer LS5 spectrofluorometer (with excitation and emission wavelengths of 550 nm and 585 nm). P450 content of cells and membranes was quantitated by the spectral method of Omura and Sato [17] using an extinction coefficient of 91 mM^{-1} cm^{-1} with an Shimazu UV-160A spectrophotometer at ambient temperature. NADPH-cytochrome *c* reduction rates were measured using an extinction coefficient of 21 mM^{-1} cm^{-1} [18].

NADPH dependence of inhibition. The bacterial membranes containing human P450 1A1 and NADPH-P450 reductase were preincubated in 100 mM potassium phosphate buffer (pH 7.4) containing resveratrol at 37°C, in the presence or absence of 120 μM NADPH [19]. At various times during the preincubation, an aliquot of the preincubation mixture was diluted 10-fold into the incubation mixture containing substrate and NADPH [20]. The product of 7-ethoxyresorufin was monitored as described above.

RESULTS

The effect of resveratrol on P450 activity was determined in human liver microsomes (HL-97 and HL-111) (Fig. 2). Resveratrol did not show any strong inhibition of EROD activity (P450 1A2 activity) in human liver microsomes (IC_{50} value 1.1 mM). To investigate the selectivity of the effect of resveratrol on P450s 1A1 and 1A2, we used *E. coli* coexpression systems containing human P450 and NADPH-P450 reductase. Interestingly, resveratrol showed strong inhibition of P450 1A1-dependent EROD and MROD activities. However, the inhibition of P450 1A2-dependent activities was not strong (Fig. 3). The IC_{50} values for CYP1A1 were 23 μM for EROD and 11 μM for MROD; those for P450 1A2 were 1.2 mM for EROD and 580 μM for MROD. Thus, resveratrol exhibited over 50-fold greater inhibition of P450 1A1 compared to P450 1A2 (Table 1).

NADPH-P450 reductase transfers electrons from NADPH to P450s, and in some cases the inhibition of P450 by chemicals is mediated via blocking of electron transfer by NADPH-P450 reductase inhibition. Resveratrol did not show any significant changes of human NADPH-cytochrome *c* reductase activity up to 100 μM (Fig. 4) and concluded that resveratrol has no effect on human P450 reductase.

To investigate the inhibition mechanism of resveratrol, the inhibition kinetic studies were performed with human P450 1A1/reductase coexpressed *E. coli* membranes (Fig. 5A). Resveratrol inhibited human P450 1A1 activity in a mixed-type inhibition (competitive-noncompetitive) with a K_i of 9.1 ± 1.9 μM (for competitive inhibition) and a K_i of 89 ± 6 μM (for noncompetitive inhibition).

To test whether resveratrol is a mechanism-based inactivator, preincubation experiments were done (Fig. 5B). The inhibition of P450 1A1 was not enhanced by preincubation for 10 min.

DISCUSSION

Resveratrol is thought to be a phytoalexin, a group of compounds that are produced during environmental stresses or pathogen attacks. It has been found in many components of human diets, such as mulberries,

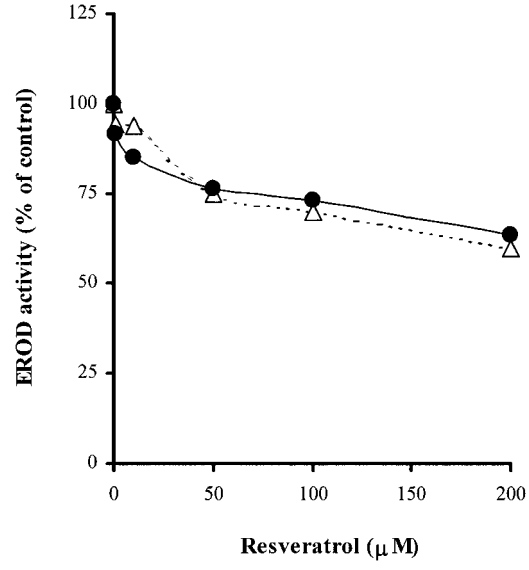

FIG. 2. Inhibition of EROD activity by resveratrol in human liver microsome samples HL-97 and HL-111. Results are expressed as a percentage of control activity in the absence of resveratrol. Each point represents the average of two independent experiments. HL-97 (△), and HL-111 (●).

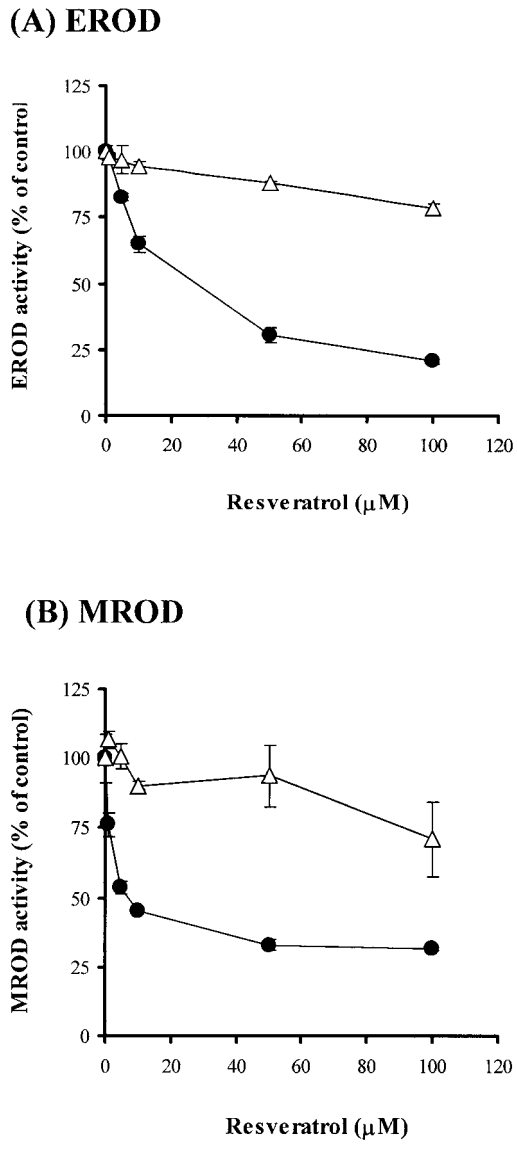

FIG. 3. Effects of resveratrol on the P450 1A-dependent monooxygenases (EROD and MROD) of *E. coli* membranes coexpressed human P450 and NADPH-P450 reductase. Each point represents the mean ± range of duplicate determinations. (A) EROD activity, (B) MROD activity. Filled circles indicate human CYP1A1/NADPH-P450 reductase coexpression, and open triangles indicate human CYP1A2/NADPH-P450 reductase coexpression.

peanuts, and grapes. The grape is a main source of resveratrol, and fresh grape skin contains about 50 to 100 μg of resveratrol per gram [21].

Recently, increasing interest has been paid to resveratrol as a cancer chemopreventive agent. It induces quinone reductase, inhibits the hydroperoxidase activity of cyclooxygenase 1, and promotes HL-60 cell differentiation. Moreover, resveratrol suppresses the formation of preneoplastic lesions in a mouse mammary grand culture and decreases the number of tumors in the two stage mouse skin cancer model [10]. Several mechanisms of anticarcinogenic effect of resveratrol were recently investigated. Resveratrol induces apoptosis and arrests the cell cycle at S/G2 phase transition [22, 23]. Nitric oxide generation and inducible nitric oxide synthase in activated macrophage with LPS were strongly suppressed by resveratrol [24, 25]. Resveratrol also inhibits cyclooxygenase 2 transcription and activity [26].

Human P450 1A1 is the principal aryl hydrocarbon hydroxylase in non-hepatic tissues and activates many environmental toxicants and procarcinogens to ultimate carcinogens [27, 28]. Although human P450 1A2 is mainly expressed in human liver, P450 1A1 is essentially an extrahepatic P450 enzyme. There is considerable evidence that P450 1A1 is expressed at both the mRNA and proteins levels in human lung [11, 29, 30]. The level of lung P450 1A1 can be induced by smoking and the metabolic activation of carcinogens in cigarette can promote lung carcinogenesis. Thus, the inhibition of P450 1A1 may be one of the key mechanisms for cancer prevention, especially for lung cancer.

Recently, the effects of resveratrol on cytochrome P450 expression were reported, but the results are controversial. Resveratrol inhibited the increase in P450 1A1 mRNA transcription caused by TCDD in cultured human HepG2 cells [31] but induced P450 1A1 mRNA in human HeLa cell cultures [32]. To elucidate the effect of resveratrol for modulating P450 activities, we evaluated resveratrol on human P450s 1A1 and 1A2 activities using human liver microsomes and bacterial membranes coexpressed human P450 and NADPH-P450 reductase [13]. This expression system has advantages including equimolar production of P450 and NADPH-P450 reductase, increased colocalization of the two proteins, and expression of full P450 activity within the bacterial membranes. Resveratrol showed selectivity for P450 1A1 over 1A2 in the coexpression system. Because human P450 1A2 is the major P450 1A subfamily protein in human liver, and EROD activity in human liver microsomes can be attributed to P450 1A2, the results from human liver microsome are quite consistent with that of recombinant human P450 1A2. Over 50-fold selectivity of resveratrol inhibition for P450 1A1 over P450 1A2 is most striking.

TABLE 1

Comparison of IC_{50} Values of Resveratrol in Expressed Human P450s 1A1 and 1A2

	IC_{50} of resveratrol (μM)*	
	EROD	MROD
P450 1A1	23	11
P450 1A2	1200	580
Ratio: P450 1A2/P450 1A1	51	51

* Each value represents the average of two independent experiments.

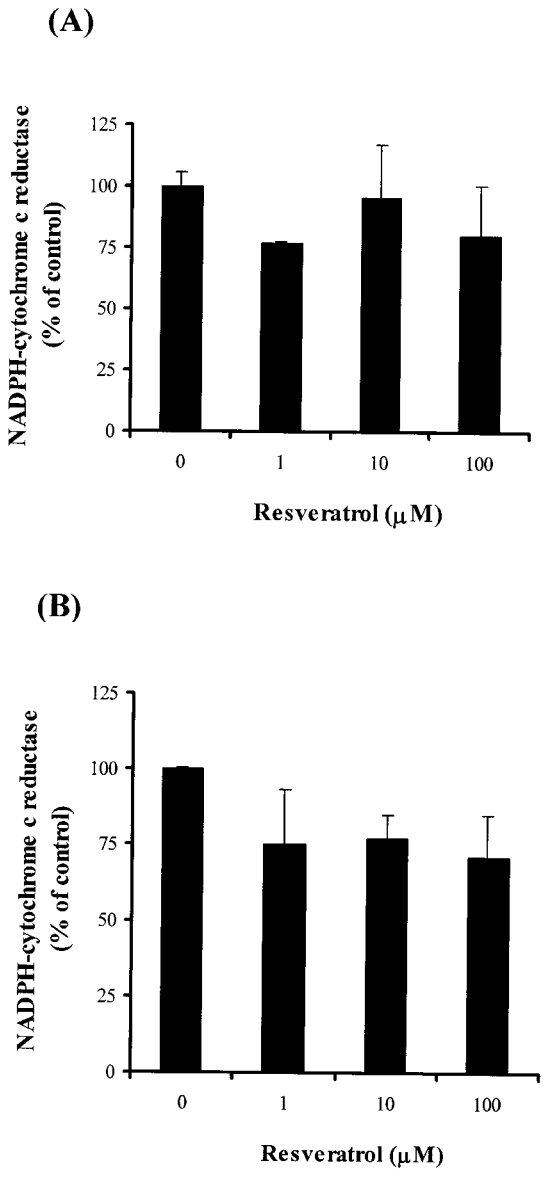

FIG. 4. Effects of resveratrol on NADPH-cytochrome *c* reductase of *E. coli* membranes in which human P450 and NADPH-P450 reductase are coexpressed. Each value represents the mean ± range of duplicate determinations. (A) human P450 1A1/NADPH-P450 reductase coexpression, (B) human P450 1A2/NADPH-P450 reductase coexpression.

The well-known P450 1A inhibitor α-naphthoflavone is selective for P450 1A2 [20]. Among various flavone compounds, galangin (3,5,7-trihydroxyflavone) exhibited 5-fold greater inhibition of P450 1A2 than of P450 1A1, and 7-hydroxyflavone showed 6-fold selectivity in its inhibition of P450 1A1 over P450 1A2 [34]. Resveratrol is one of the most selective natural inhibitors of human P450 1A1.

We determined the enzyme kinetics of resveratrol inhibition. Resveratrol showed mixed-type inhibition (competitive-noncompetitive) of human P450 1A1. It is not a mechanism-based inactivator of P450 1A1. These results suggest that resveratrol competes for substrate binding site with ethoxyresorufin while it can bind at a different site from the substrate, although exactly how this occurs is not clear.

Based on our results, we report for the first time that resveratrol is a selective human P450 1A1 inhibitor.

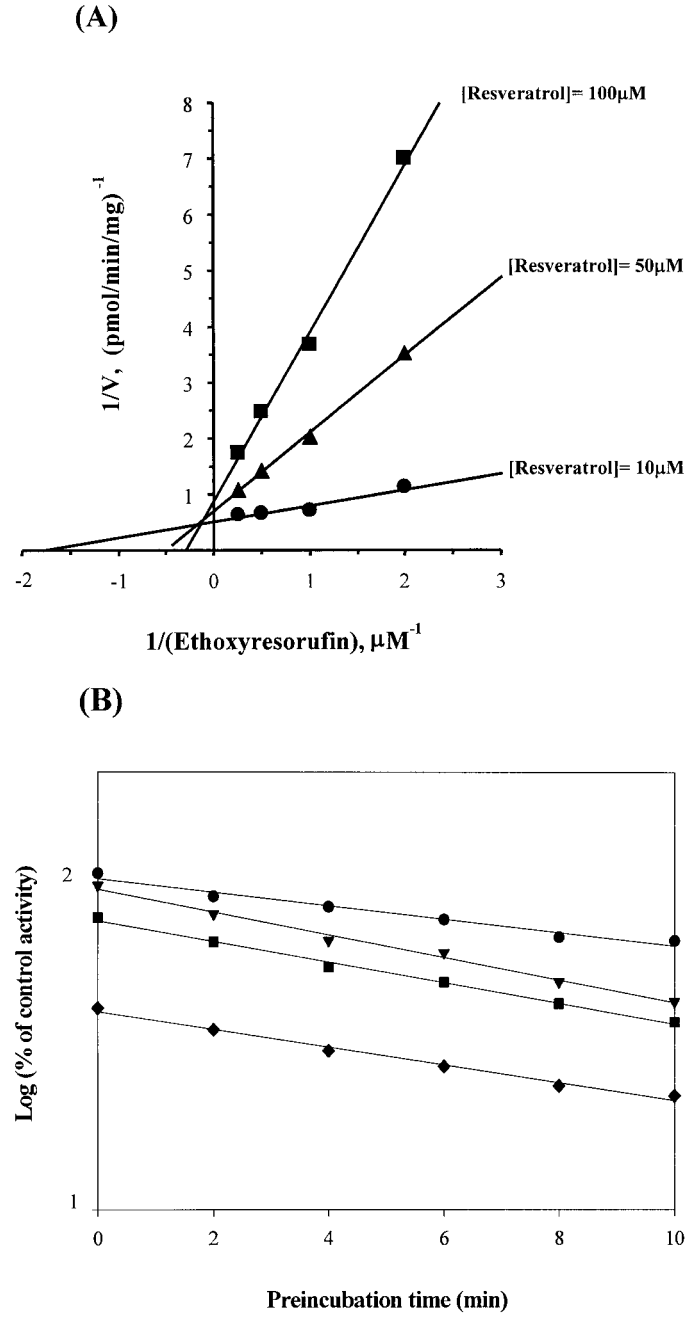

FIG. 5. Inhibition kinetics of human P450 1A1 activity (EROD) by resveratrol. (A) Lineweaver-Burk plot in the presence of resveratrol by expressed P450 1A1. (B) Effect of preincubation on the inhibition of P450 1A1-dependent EROD by resveratrol. At $t = 0$, no inhibitor (●) or 10 μM (▼) or 50 μM (■) or 100 μM (◆) resveratrol was added to an preincubation mixture containing NADPH.

The inhibitory effect of resveratrol on human P450 1A1 may be mainly involved in protecting against tumor formations and carcinogenesis.

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