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MECHANISM-BASED INHIBITION OF HUMAN CYTOCHROME P450 1A1 BY RHAPONTIGENIN

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ABSTRACT:

Recently we reported that resveratrol (*trans*-3,4′,5-trihydroxystilbene) showed selective inhibition of recombinant human cytochrome P450 (P450) 1A1 in a concentration-dependent manner. The inhibition of recombinant human P450 1A1, 1A2, or 1B1 by various hydroxystilbene compounds having a similar structure to resveratrol was investigated using bacterial membranes from a human P450/NADPH-P450 reductase bicistronic expression system to find new candidates for cancer chemopreventive agents. Of seven compounds tested, rhapontigenin (3,3′,5-trihydroxy-4′-methoxystilbene) exhibited a potent and selective inhibition of human P450 1A1 with an IC_{50} value of 0.4 μM. Rhapontigenin showed 400-fold selectivity for P450 1A1 over P450 1A2 and 23-fold selectivity for P450 1A1 over P450 1B1. Rhapontigenin did not show any significant inhibition of ethoxyresorufin *O*-deethylation (EROD) activity in human liver microsomes, the other human P450s such as P450 2E1, P450 3A4, P450 2D6, P450 2C8, and P450 2C9, or human NADPH-P450 reductase. We have further investigated the inhibition kinetics of P450 1A1 by rhapontigenin. Rhapontigenin inhibited EROD activity of expressed human P450 1A1 in a competitive manner. The loss of EROD activity was time- and concentration-dependent. The values for K_i and $k_{inactivation}$ were 0.09 μM and 0.06 min^{-1}, respectively. The loss was not blocked by the trapping agents glutathione, *N*-acetylcysteine, or dithiothreitol. These results suggest that rhapontigenin is a potent mechanism-based inactivator of human P450 1A1 and may be considered as a good candidate for a cancer chemopreventive agent in humans.

Human cytochrome P450 (P450[1]) 1A1 is a well known aryl hydrocarbon hydroxylase and is involved in the metabolic activation of procarcinogens of the polycyclic aromatic hydrocarbons. In humans, P450 1A1 shares 80% amino acid sequence identity with P450 1A2 and about 40% with P450 1B1 (Jaiswal et al., 1985; Quattrochi et al., 1985; Shimada et al., 1998b). The substrate specificities of these enzymes can often overlap. Although P450 1A1 is mainly expressed in human lung, placenta, and lymphocytes, P450 1A2 is one of the major P450s in human liver. P450 1B1 is normally expressed in human fibroblasts and steroidogenic tissues. To distinguish the activities of these P450s and to determine the mechanism of reactions of the enzymes, selective inhibitors are required. Selective inhibitors may be good chemopreventive agent candidates for cancer because these P450s are related to tumor initiation.

Resveratrol has been studied to determine its role as a cancer chemopreventive agent and shown to inhibit tumor formation, acting through cyclooxygenase inhibition, blockage of free-radical formation, and induction of quinone reductase (Jang et al., 1999).

Recently we reported that resveratrol can inhibit human P450 1A1 in a concentration-dependent manner (Chun et al., 1999). Resveratrol showed 50-fold selectivity in its inhibition of P450 1A1 over P450 1A2. However, the IC_{50} value for 1A1 inhibition (23 μM) by resveratrol is not very low compared with other known P450 1A inhibitors such as α-naphthoflavone and 7-hydroxyflavone (Shimada et al., 1998b; Zhai et al., 1998). To find more potent and selective P450 1A1 inhibitors among resveratrol analogs, several hydroxystilbene compounds obtained from natural sources were evaluated for selective inhibition of P450 1A1 activity (Shin et al., 1998). To examine these compounds, recombinant systems in which human P450s 1A1, 1A2, or 1B1 was expressed along with human NADPH-P450 reductase in bacterial membranes were used (Parikh et al., 1997; Josephy et al., 1998; Shimada et al., 1998a). Of the compounds tested, rhapontigenin showed potent and selective inhibition of P450 1A1 over 1A2 or 1B1. Kinetic analyses were performed to determine the mechanism of P450 1A1 inhibition, and we suggest that rhapontigenin is a mechanism-based inactivator of human P450 1A1.

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[1] Abbreviations used are: P450, cytochrome P450; EROD, ethoxyresorufin *O*-deethylation; IPTG, isopropyl-1-thio-β-D-galactopyranoside.

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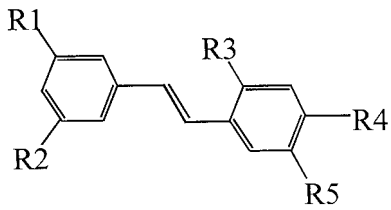
Experimental Procedures

Materials. The hydroxystilbenes used were isolated from herbal extracts or obtained from chemical modification (Table 1) (Ryu et al., 1988). Rhapontigenin, rhaponticin, and 3,5-dihydroxy-4′-methoxystilbene were purified from *Rheum undulatum* Linne. Oxyresveratrol was isolated from *Morus alba* Linne. Resveratrol was isolated from *Veratrium album* var. *grandiflorum* Maxim., and 3,4′,5-trimethoxystilbene was obtained by methylation of resveratrol. Piceid was isolated from *Polygonum cuspidatum* Sieb. et Zucc., and 3,4′-dimethoxy-5-hydroxystilbene was obtained by methylation of piceid followed by the acid hydrolysis. Human liver samples were kindly provided by Dr. F. Peter Guengerich (Vanderbilt University, Nashville, TN). Ethoxyresorufin, resorufin, ethoxycoumarin, hydroxycoumarin, dimethyl sulfoxide, thiamine, IPTG,

TABLE 1

Structure of hydroxystilbenes used in this study



Compound	Substitution				
	R1	R2	R3	R4	R5
Rhapontigenin	OH	OH	H	OCH_3	OH
3,5-Dihydroxy 4′-methoxystilbene	OH	OH	H	OCH_3	H
Oxyresveratrol	OH	OH	OH	OH	H
3,4′-Dimethoxy-5-hydroxystilbene	OH	OCH_3	H	OCH_3	H
3,4′,5-Trimethoxystilbene	OCH_3	OCH_3	H	OCH_3	H
Piceid	*O*-Glucose	OH	H	OH	H
Rhaponticin	*O*-Glucose	OH	H	OCH_3	OH

δ-aminolevulinic acid, and NADPH were purchased from Sigma Chemical Co. (St. Louis, MO). Nifedipine and nifedipine metabolite were from RBI/Sigma (Natick, MA). Bactopeptone, yeast extract, and bacto-agar were obtained from Difco Lab. (Detroit, MI). Human P450 2C8 microsomes were purchased from GENTEST (Woburn, MA). Other chemicals were of the highest grade commercially available.

Recombinant Human P450s. Coexpression (bicistronic) plasmids for human P450s (1A1, 1A2, 1B1, 2E1, 3A4, 2C9, or 2D6), and NADPH-P450 reductase were transformed into *Escherichia coli* DH5α (Parikh et al., 1997). A single ampicillin-resistant colony of transformed cells was selected and grown in overnight culture to saturation at 37°C in Luria-Bertani medium containing 100 μg of ampicillin ml^{-1}. A 10-ml aliquot was used to inoculate each liter of Terrific Broth containing 0.2% bactopeptone (w/v), 100 μg of ampicillin ml^{-1}, 1.0 mM thiamine, trace elements, 0.5 mM δ-aminolevulinic acid, and 1.0 mM IPTG. The cultures were grown at 30°C with shaking at 200 rpm for 24 h. Membrane fractions were prepared by differential centrifugation from bacteria and suspended in 10 mM Tris-HCl buffer (pH 7.4) containing 1.0 mM EDTA and 20% (v/v) glycerol (Guengerich et al., 1996).

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 10^4g for 20 min at 4°C, and the resulting supernatant was centrifuged at 10^5g for 60 min at 4°C. The microsomal pellets were resuspended in 10 mM Tris acetate buffer (pH 7.4) containing 1.0 mM EDTA and 20% (v/v) glycerol (Guengerich, 1994). Protein concentrations were estimated using the bicinchoninic acid method according to the supplier's recommendations (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as a standard. The isolated microsomes were stored at −80°C.

Enzyme Assays. EROD activity was determined for the measurement of P450 1A1, 1A2, or 1B1 activities (Burke et al., 1985). The reaction mixture contained 0.1 M potassium phosphate buffer (pH 7.4), 2 mg of bovine serum albumin ml^{-1}, 10 μM dicoumarol, human liver microsomes, or *E. coli* membranes (5 nM P450 1A1, 10 nM P450 1A2, or 10 nM P450 1B1, respectively), and 2 μM ethoxyresorufin. The reaction mixtures were preincubated at 37°C for 3 min, and the reaction was initiated by addition of 120 μM NADPH. Incubations were performed in a shaking water bath for 20 min at 37°C and terminated by addition of 1 ml of methanol. The formation of resorufin was determined fluorometrically with a PerkinElmer LS 5 spectrofluorometer (with excitation and emission wavelengths of 550 and 585 nm, respectively) (PerkinElmer, Norwalk, CT).

P450 content of cells and membranes was quantitated by the spectral method of Omura and Sato (1964) using an extinction coefficient of 91 mM^{-1} cm^{-1} with a Shimadzu UV-160A spectrophotometer at ambient temperature (Shimadzu, Kyoto, Japan). For the measurement of P450 2E1 activity, *O*-deethylation of 7-ethoxycoumarin was determined fluorometrically according to methods described previously (Yamazaki et al., 1996). Reaction mixtures contained 0.1 M potassium phosphate (pH 7.4), 2 mM ethoxycoumarin, *E. coli* membranes (40 nM P450 2E1), and 1 mM NADPH. Incubations were performed in a shaking water bath for 20 min at 37°C and terminated by addition of 25 μl of 10% (w/v) trichloroacetic acid and 1 ml of CH_2Cl_2. The mixture was vortex mixed and extracted with 30 mM sodium borate (pH 9.0). Concentrations of hydroxycoumarin were determined by a PerkinElmer LS 5 spectrofluorometer with excitation and emission wavelengths of 370 and 450 nm, respectively.

Nifedipine oxidation was determined by high-performance liquid chromatography as a measure of recombinant human P450 3A4 activity using previously described methodology (Guengerich et al., 1986). Reaction mixtures contained 50 mM HEPES (pH 7.4), 30 mM $MgCl_2$, 0.2 mM nifedipine, *E. coli* membranes (40 nM P450 3A4), and 1 mM NADPH. Incubations were performed in a shaking water bath for 20 min at 37°C and terminated by addition of 2 ml of CH_2Cl_2 and 100 μl of 1 M Na_2CO_3 (pH 10.5) containing 2 M NaCl. The samples were centrifuged at 3000*g* for 10 min, and 1.4 ml of each of the lower organic layers were dried under a N_2 stream. Determination of a nifedipine metabolite was performed using a 150- × 4.6-mm steel C_{18} Nucleosil column with ultraviolet detection at 254 nm. The flow rate was 1.0 ml/min, and the mobile phase was 64% methanol. Under these conditions, retention times of nifedipine metabolite and nifedipine were 6.9 and 9.1 min, respectively. The detection limit of a nifedipine metabolite was ~50 pmol with a signal-to-noise ratio of 3:1. Bufuralol 1′-hydroxylation activity was determined as previously described (Yamazaki et al., 1994). Incubation mixtures consisted of membranes (100 nM P450 2D6) and bufuralol (0.4 mM) in a final volume of 0.1 ml of 100 mM potassium phosphate buffer (pH 7.4) containing 1 mM NADPH. Incubations were carried out at 37°C for 20 min and were terminated by addition of 10 μl of 60% (w/v) $HClO_4$. The mixtures were centrifuged at 3000*g* for 5 min, and aliquots of the supernatant were separated using a 150- × 4.6-mm steel C_{18} Nucleosil column eluted with 45% (v/v) acetonitrile/55% 20 mM $NaClO_4$, pH 2.5. The hydroxylated metabolites of bufuralol were detected with a fluorescence detector (excitation = 252 nm; emission = 302 nm).

Paclitaxel 6α-hydroxylase activity was used as a catalytic marker for recombinant human P450 2C8 (Rahman et al., 1994). Reaction mixtures (0.2 ml of total volume) contained 100 mM potassium phosphate buffer (pH 7.4), 10 μM paclitaxel, membranes (50 nM P450 2C8), and 1 mM NADPH. Incubations were performed in a shaking water bath for 20 min at 37°C and terminated by addition of 50 μl of ice-cold acetonitrile. The samples were centrifuged at 12,000*g* for 5 min, and aliquots of the supernatant were analyzed using a 150- × 4.6-mm steel C_{18} Nucleosil column with a linear gradient of 45% of 10% (v/v) methanol and 55% of 100% (v/v) methanol to 35% of 10% (v/v) methanol and 65% of 100% (v/v) methanol over 20 min, then for a further 5 min with 35% of 10% (v/v) methanol and 65% of 100% (v/v) methanol with ultraviolet detection at 254 nm.

Diclofenac 4′-hydroxylation was determined for the recombinant human P450 2C9 specific activity (Leemann et al., 1992). Incubation mixture (0.2 ml of total volume) consisted of 100 mM Tris-HCl buffer (pH 7.5), 100 μM diclofenac, membranes (50 nM P450 2C9), and 1 mM NADPH. Incubations were carried out at 37°C for 20 min and were terminated by addition of 50 μl of a mixture of 94% acetonitrile/6% acetic acid. The reaction mixtures were centrifuged at 12,000*g* for 5 min and the supernatant was analyzed using a 150- × 4.6-mm steel C_{18} Nucleosil column with a linear gradient from 70% of 30% (v/v) acetonitrile containing 2 mM perchloric acid and 30% of 100% (v/v) methanol to 100% of 100% (v/v) methanol over 20 min. The flow rate was 1.0 ml/min, and the production of 4′-hydroxydiclofenac was measured with ultraviolet detection at 280 nm.

Rates of NADPH-cytochrome *c* reduction were measured using an extinction coefficient of 21 mM^{-1} cm^{-1} (Yasukoshi and Masters, 1976). The intra- and interday variation coefficients did not exceed 10% in any of the assays.

NADPH Dependence of Inhibition. Bacterial membranes containing human P450 1A1 and NADPH-P450 reductase were preincubated in 0.1 M potassium phosphate buffer (pH 7.4) containing hydroxystilbene at 37°C for 10 min in the presence or absence of 1 mM NADPH (Chun et al., 1999). At various times during the preincubation, an aliquot of the preincubation mixture was diluted 10-fold into the reaction mixture containing 0.1 M potassium phosphate (pH 7.4), 2 mg/ml bovine serum albumin, 0.2 μM ethoxyresorufin, and 120 μM NADPH. The mixtures were further incubated at 37°C for 20 min.

TABLE 2

Inhibition of human P450 1A1, 1A2, and 1B1 by various hydroxystilbene compounds

P450 concentrations for determination of catalytic activities were 5, 10, and 10 nM for P450 1A1, 1A2, and 1B1, respectively. Control activities (means of triple determinations) in the absence of chemicals were 12.5, 5.4, and 2.5 nmol of resorufin formed min^{-1} nmol of $P450^{-1}$ for P450 1A1, 1A2, and 1B1.

Compound	IC_{50}			Ratio (1A2/1A1)	Ratio (1B1/1A1)
	1A1	1A2	1B1		
		μM			
Rhapontigenin	0.4	160	9	400	23
3,5-Dihydroxy 4′-methoxystilbene	0.4	35	1	88	3
Oxyresveratrol	15	150	34	10	2
3,4′-Dimethoxy-5-hydroxystilbene	0.1	8	0.1	80	1
3,4′,5-Trimethoxystilbene	0.6	0.6	0.4	1	0.7
Piceid	480	>2000	>1000		
Rhaponticin	200	166	145	0.8	0.7

The product of 7-ethoxyresorufin was monitored fluorometrically as described above.

Data Analysis. Kinetic parameters from individual experiments were calculated using a nonlinear regression analysis program (Prism, GraphPad Software, San Diego, CA).

Results

Inhibition of EROD activity by Hydroxystilbenes. To examine their effects on the activity of human P450 1A1, 1A2, and 1B1, seven hydroxystilbene compounds were compared with respect to inhibition of EROD activities (Table 2). Rhapontigenin was found to be a potent inhibitor of P450 1A1, with an IC_{50} value of 0.4 μM (Fig. 1; Table 2). Similar studies showed that rhapontigenin also inhibits P450 1A2 and 1B1, but the IC_{50} values were much higher (i.e., 160 μM for P450 1A2 and 9 μM for P450 1B1). Thus, rhapontigenin exhibited 400-fold greater selective inhibition of P450 1A1 over 1A2 and 23-fold greater inhibition of P450 1A1 over 1B1. As shown in Table 2, 3,4′-dimethoxy-5-hydroxystilbene and 3,5-dihydroxy-4′-methoxystilbene were also more potent inhibitors of P450 1A1 than of P450 1A2, but the selectivity between P450 1A1 and 1B1 was much lower than that of rhapontigenin. The most potent P450 1A1 inhibitor of all hydroxystilbenes we examined was 3,4′-dimethoxy-5-hydroxystilbene. 3,4′,5-Trimethoxystilbene also strongly inhibited P450 1A1, 1A2, and 1B1 with IC_{50} values of 0.6, 0.6, and 0.4 μM for 1A1, 1A2, and 1B1, respectively, but no selectivity was shown. The IC_{50} of oxyresveratrol for P450 1A1 was about 15 μM. The hydroxystilbenes containing a glucosyl moiety at the R1 position, such as piceid or rhaponticin, had little inhibitory effect on P450s. The bulky and polar glucosyl moiety may block the accessibility of the chemicals into the active sites of P450s.

Rhapontigenin showed very weak inhibition of the catalytic activities of the other human P450s, such as P450 2E1, 3A4, 2D6, 2C8, and 2C9 (Fig. 1). The EROD activity in human liver microsomes and human NADPH-P450 reductase activity of *E. coli* membrane coexpressing human P450 1B1 and NADPH-P450 reductase also were not significantly changed by rhapontigenin (Fig. 2).

Mechanism of Inhibition by Rhapontigenin. To examine the mechanism of inhibition by rhapontigenin, kinetic studies were performed using recombinant human P450 1A1. Rhapontigenin showed competitive inhibition of P450 1A1, with a K_i of 0.21 μM (Fig. 3). Preincubation of P450 1A1 with various concentrations of rhapontigenin in the presence of NADPH resulted in a time- and concentration-dependent loss of EROD activity. The inactivation of P450 1A1 by rhapontigenin exhibited first order kinetics when plotted as the log of percentage of activity remaining versus time (Fig. 4A). A replot of

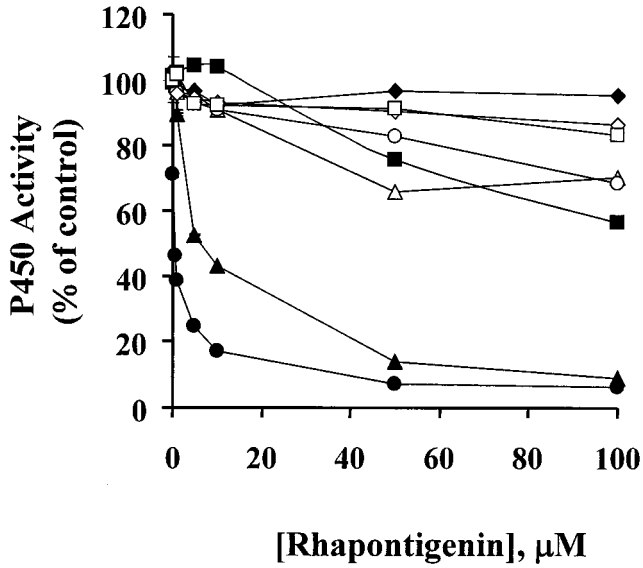


FIG. 1. *Effects of rhapontigenin on the P450 1A1, 1A2, 1B1, 2E1, 3A4, 2D6, 2C8, or 2C9 activities in the P450-expression systems.*

Membranes coexpressing human P450s and NADPH-P450 reductase were incubated with rhapontigenin for 20 min at 37°C in the presence of NADPH. Assays included EROD by P450 1A1 (●), P450 1A2 (■), or 1B1 (▲), 7-ethoxycoumarin *O*-deethylation by P450 2E1 (♦), nifedipine oxidation by P450 3A4 (△), bufuralol 1′-hydroxylation by P450 2D6 (○), paclitaxel 6α-hydroxylase activity by P450 2C8 (◇), or diclofenac 4′-hydroxylation by P450 2C9 (□) in the presence of the indicated concentrations of rhapontigenin. Each data point represents the mean ± S.E.M. of three experiments.

the data in Fig. 4A showed that K_i and $k_{inactivation}$ were 0.09 μM and 0.06 min^{-1}, respectively (Fig. 4B). To determine the possibility that the reactive species could escape from the P450 active site and bind to nucleophilic sites in the vicinity of the active site, the effect of trapping agents such as glutathione, *N*-acetylcysteine, or dithiothreitol on the inactivation was studied. Rhapontigenin did not protect against P450 1A1 inactivation in the presence of 2 mM glutathione, *N*-acetylcysteine, or dithiothreitol (Fig. 5).

Discussion

Tumor initiation begins when DNA in cells is damaged by exposure to carcinogens. If this damage is not repaired, it can lead to genetic mutations (Hurting et al., 1999). Metabolic activation of procarcinogens is often catalyzed by P450 enzymes through oxidation. Enzymes such as P450 1A1, 1A2, or 1B1 of the human P450 1 subfamily are responsible for much of the metabolism of procarcinogens and regarded as the target enzymes for blocking tumor initiation (Shimada et al., 1989, 1996; Guengerich and Shimada, 1991). Therefore, specific P450 inhibitors or inactivators could be beneficial for preventing tumor formation.

Recently, several hydroxystilbene compounds isolated from herbal sources including *Morus alba* or *Rheum undulatum* were identified as having various pharmacological effects. For example, oxyresveratrol potently inhibited dopa oxidase activity of tyrosinase (Shin et al., 1998a). Rhapontigenin significantly inhibited the release of β-hexaminidase from cultured RBL-2H3 cells (Cheong et al., 1999). These hydroxystilbenes also suppressed ovine cyclooxygenase-1 activity (Shin et al., 1998b).

In this study we demonstrated that rhapontigenin is a potent mechanism-based inactivator of P450 1A1 as well as a competitive inhibitor. Rhapontigenin inactivation of P450 1A1 followed pseudo-first order kinetics, was time- and concentration-dependent, and required NADPH. Trapping agents such as glutathione, *N*-acetylcysteine, or

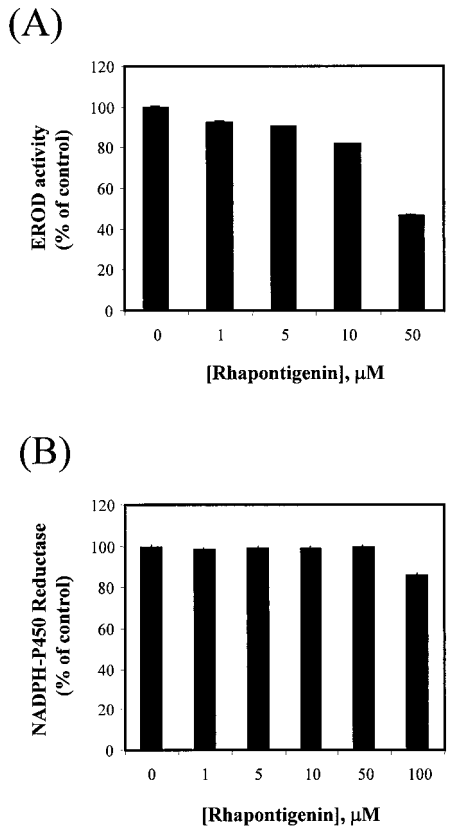


FIG. 2. *Effects of rhapontigenin on EROD activity catalyzed by human liver microsomes of human sample HL-97 (A) and NADPH-P450 reductase activity in E. coli membrane coexpressing human P450 1A1 and NADPH-P450 reductase (B).*

Each data point represents the mean ± range of duplicate determinations.

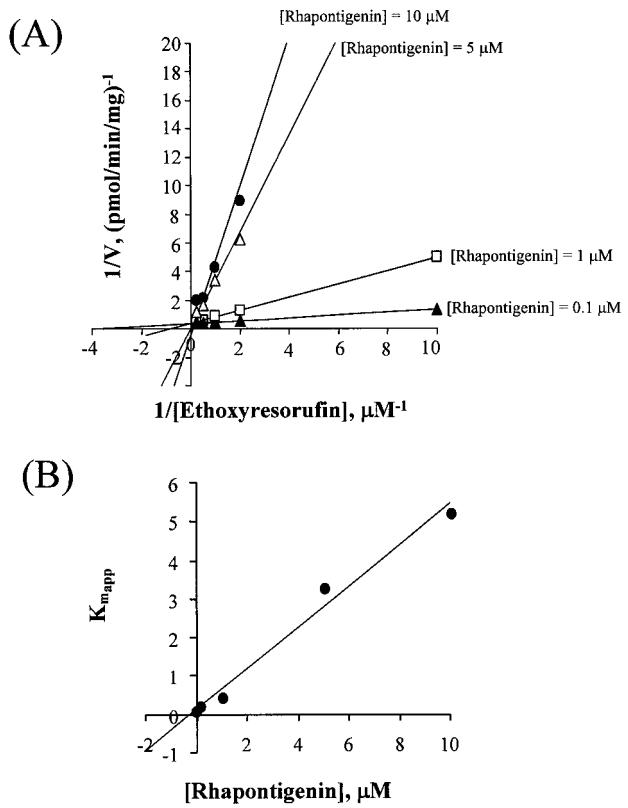


FIG. 3. *Kinetic analysis of human P450 1A1 inhibition by rhapontigenin.*

EROD activity was determined with *E. coli* membrane coexpressed human P450 1A1 and NADPH-P450 reductase. A, Lineweaver-Burk plot in the presence of rhapontigenin. B, replot of the data from the Lineweaver-Burk plot indicating that the K_i for rhapontigenin is 0.21 μM.

dithiothreitol could not block the inactivation of P450 1A1 by rhapontigenin.

Several chemopreventive agents are known as a mechanism-based inactivators of P450s, such as isothiocyanates (P450 2E1) and oltipraz (P450 1A2) (Kent et al., 1998; Moreno et al., 1999; Langouët et al., 2000). The two main mechanisms for the chemopreventive action are 1) inhibition of phase I enzymes such as the P450 enzymes and 2) induction of phase II enzymes such as glutathione *S*-transferase and quinone reductase. We have previously demonstrated that human P450 1A1 activity was selectively inhibited by resveratrol (Chun et al., 1999). Resveratrol seems to be a promising cancer chemopreventive agent because it inhibits phase I enzymes and induces phase II enzymes. However, the inhibition of P450 1A1 by resveratrol is relatively weak and not mechanism-based. Furthermore, resveratrol could not discriminate in inhibiting the activities of P450s 1A1 and 1B1 (unpublished results). We suggest that rhapontigenin is a better candidate for cancer chemopreventive agents because its inhibitory potential of P450 1A1 is much stronger, selective, and mechanism-based. We are studying the effects of rhapontigenin on the expression of phase II enzymes in cultured cells.

A number of the known inhibitors of the different P450 enzymes are mechanism-based (Yun et al., 1992; Wrighton et al., 1993; Roberts et al., 1995; Heyn et al., 1996; Hickman et al., 1998). Many selective P450 1A1 inhibitors have been reported. For example, 2-(1-propynyl)phenanthrene showed 70-fold greater inhibition of P450 1A1 than of 1A2 (Shimada et al., 1998b), and 7-hydroxyflavone exhibited 6-fold greater selectivity in its inhibition of P450 1A1 over P450 1A2 (Zhai et al., 1998). As we showed previously, resveratrol is also selective for inhibiting P450 1A1 (Chun et al., 1999). These

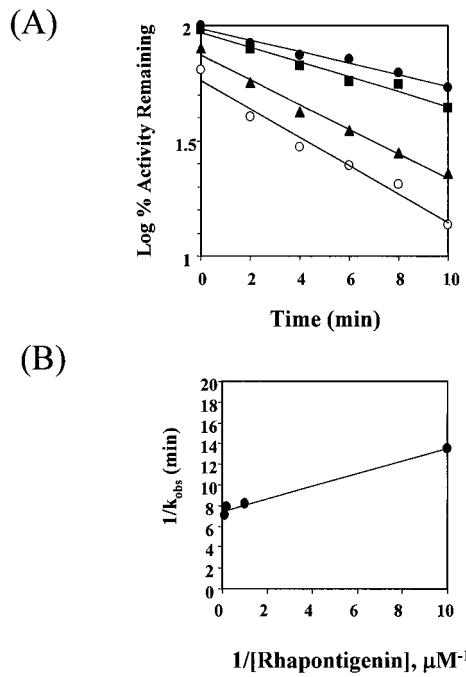


FIG. 4. *Mechanism-based inactivation of human P450 1A1 by rhapontigenin.*

The concentrations of rhapontigenin were 0 (●), 0.1 (■), 1 (▲), and 10 μM (○). A, time- and concentration-dependent loss of P450 1A1 activity with rhapontigenin and NADPH. B, the double reciprocal plot of the rates of inactivation as a function of the rhapontigenin concentration. The values for $1/k_{obs}$ (min^{-1}) were calculated from the inactivation experiments shown in A.

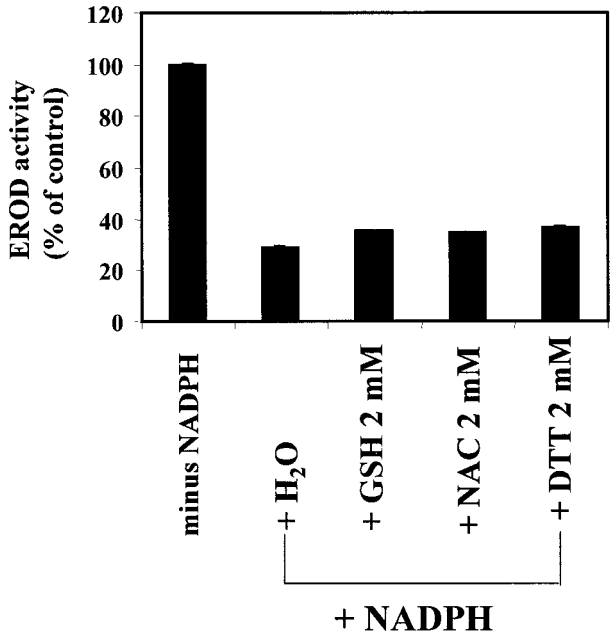


FIG. 5. *Effects of the trapping agents on the inactivation of P450 1A1 by rhapontigenin.*

The *E. coli* membranes expressing human P450 1A1 and NADPH-P450 reductase were preincubated for 10 min with NADPH (1 mM), rhapontigenin (5 μM), and the indicated trapping agents (2 mM) or water. At the end of the incubation, aliquots were assayed for EROD activity. Each data point represents the mean ± range of duplicate determinations.

inhibitors are used for labeling the P450 active site and identifying critical amino acid(s) of P450s. The 400-fold greater selectivity of rhapontigenin in the inhibition of P450 1A1 over P450 1A2 is of interest. Thus far, rhapontigenin is one of the most selective inhibitors of P450 1A1 from natural sources. We propose that rhapontigenin also will be a useful compound for characterizing P450 1A1 active sites because of its strong selectivity.

The precise mechanism by which rhapontigenin is transformed by P450 1A1 into an inactivating intermediate is not yet known. We have considered the possibility that activated oxygen bound to heme may react with the allyl moiety of the stilbene structure, and the intermediate species could then react with protein or heme nucleophiles in a manner that destroys heme function or binding (Ortiz de Montellano and Correia, 1983; Hammons et al., 1989; Guengerich, 1990).

In summary, we report for the first time that rhapontigenin is a potent mechanism-based inactivator of human P450 1A1. Because rhapontigenin is isolated from the oriental medical plant *Rheum undulatum*, its potent and selective inhibitory effect on P450 1A1 takes on additional advantages as a chemopreventive agent. Future studies will focus on the potential of rhapontigenin as a strong chemopreventive agent in vivo.

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