Different Mechanisms for Inhibition of Human Cytochromes P450 1A1, 1A2, and 1B1 by Polycyclic Aromatic Inhibitors

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · APRIL 2007

Impact Factor: 3.53 · DOI: 10.1021/tx600299p · Source: PubMed

6 AUTHORS, INCLUDING:

Kazushi Okada
South University of Science and Technology …
16 PUBLICATIONS **264** CITATIONS
SEE PROFILE

Hiroshi Yamazaki
Showa Pharmaceutical University
400 PUBLICATIONS **12,282** CITATIONS
SEE PROFILE

Different Mechanisms for Inhibition of Human Cytochromes P450 1A1, 1A2, and 1B1 by Polycyclic Aromatic Inhibitors

Tsutomu Shimada,*,† Norie Murayama,‡ Kazushi Okada,† Yoshihiko Funae,† Hiroshi Yamazaki,‡ and F. Peter Guengerich§

Department of Chemical Biology, Osaka City University Medical School, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan, Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan, and Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232-0146

Received October 31, 2006

We have previously shown that several polycyclic aromatic hydrocarbons (PAHs) strongly inhibit their own and other PAH metabolism catalyzed by cytochrome P450 (P450) 1A1, 1A2, and 1B1 [Shimada, T., and Guengerich, F. P. (2006) *Chem. Res. Toxicol. 19*, 288−294]. In the present study, we examined mechanisms of how PAHs inhibit these P450 enzymes by using 7-ethoxyresorufin *O*-deethylation (EROD) as a model reaction. First, we examined mechanisms of inhibition of P450 1A1, 1A2, and 1B1 by the synthetic model inhibitors 1-(1-propynyl)pyrene (1PP), 1-ethynylpyrene (1EP), 2-ethynylpyrene (2EP), and 4-(1-propynyl)biphenyl (4Pbi). Both 1PP and 1EP inhibited P450 1A1 in a mechanism-based manner, but P450 1B1 and 1A2 were directly inhibited by 1PP and 1EP. Interestingly, P450 1B1 inactivated 1PP and 1EP to products that were not inhibitory to P450 1B1. 4Pbi was a mechanism-based inhibitor of P450 1A1 and 1B1, but 2EP directly inhibited these P450s. All four of the inhibitors directly inhibited P450 1A2. We also found that benzo[*a*]pyrene and seven other PAH compounds tested inhibited P450 1A2 in a mechanism-based manner, but fluoranthene directly inhibited P450 1A2. All of the nine PAHs examined were direct inhibitors of P450 1A1 and P450 1B1. These results suggest different mechanisms of inhibition of P450 1A1, 1A2, and 1B1 by PAHs and related chemicals and that interactions between P450 enzymes and PAH inhibitors are involved in differences in inhibition of the enzymes.

Introduction

Polycyclic aromatic hydrocarbons (PAHs)[1] are ubiquitous environmental carcinogens and have been shown to be carcinogenic only after metabolic activation by so-called xenobiotic-metabolizing enzymes such as P450 (*1, 2*). P450 1A1 and 1B1 are the major enzymes involved in activating PAHs to reactive products that initiate transformation to produce tumors (*3, 4*). P450 1A2 oxidizes PAHs at slower rates than P450 1A1 and 1B1 and has been shown to be one of the major enzymes that activate aryl- and heterocyclic amines to carcinogenic products (*5*). There are several types of inhibitors of P450 1A1, 1A2, and 1B1, and some of these inhibitors have been shown to prevent formation of tumors caused by various environmental carcinogens (*6*−*8*).

We have recently shown that many PAH compounds, several of which are carcinogenic but others that are weak or inactive carcinogens in rodents (*1*), inhibit P450 1A1, 1A2, and 1B1 very strongly (*9*). The results are of interest because these PAHs inhibit their own and other PAH metabolism, possibly by influencing their carcinogenic potential (*9*−*11*). For example, benzo[*a*]pyrene (B[*a*]P) and 5-methylchrysene (5MeCh) inhibit P450 1B1-dependent 7-ethoxyresorufin *O*-deethylation (EROD) activity at nanomolar concentrations, and these PAHs also inhibit metabolic activation of B[*a*]P-7,8-diol and 5MeCh-1,2-diol to DNA-damaging products that induce *umu* gene expression in a *Salmonella typhimurium* NM2009 tester strain (*9*). The inhibition of P450 activities by PAHs varies depending on the P450 enzymes used and the PAH chemicals examined (*9*). However, how these PAHs inhibit P450 activities is not fully understood.

In this study, we examined mechanisms of inhibition of human P450 1A1, 1A2, and 1B1 by PAHs by measuring EROD, a model activity, in a mechanism-based manner. P450 enzymes used were from membranes of *Escherichia coli* in which modified P450 cDNA and human NADPH-P450 reductase cDNA have been introduced. We first examined the mechanism of inhibition of P450s by the synthetic inhibitors 1-(1-propynyl)-pyrene (1PP), 1-ethynylpyrene (1EP), 2-ethynylpyrene (2EP), and 4-(1-propynyl)biphenyl (4Pbi). The results are of interest because 1PP and 1EP inhibit P450 1A1 in a mechanism-based manner, but they inhibit P450 1A2 and 1B1 directly, in a competitive manner. P450 1B1 was also found to oxidize 1PP and 1EP to products that lose activity as P450 1B1 inhibitors. The mechanisms of inhibition of P450 1A1, 1A2, and 1B1 by various PAH compounds were examined further.

Experimental Procedures

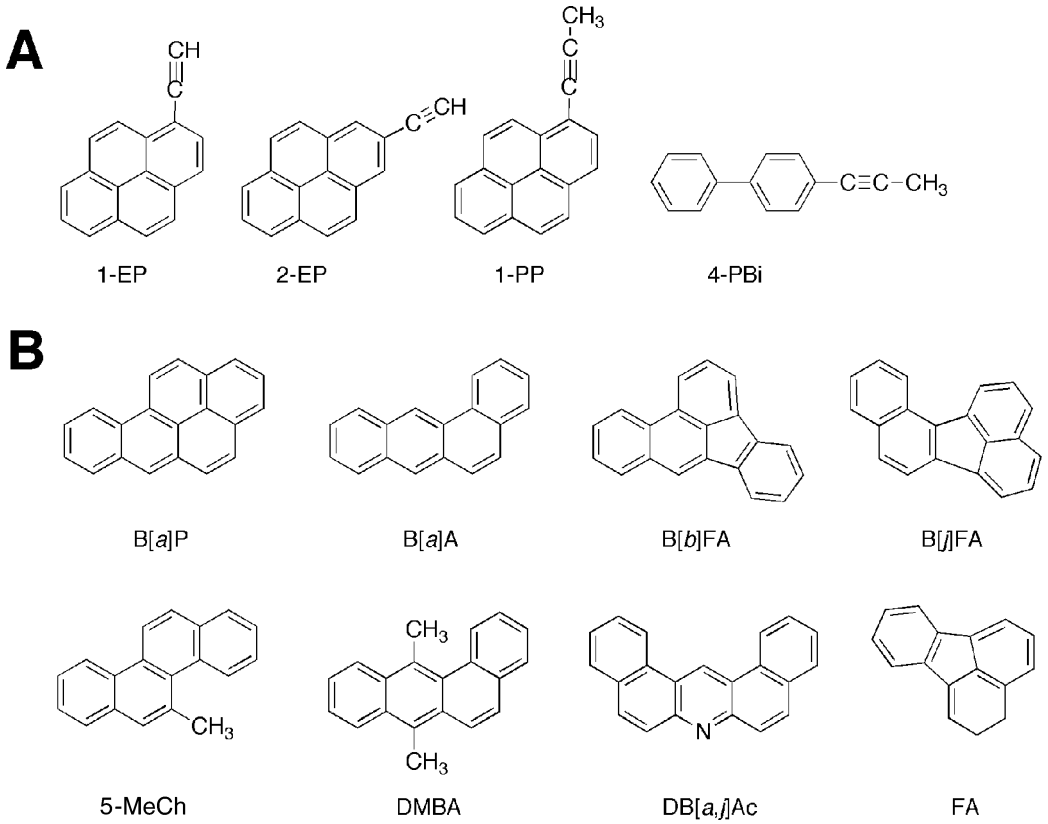
Chemicals. 1EP, 1PP, 2EP, and 4Pbi (Scheme 1) were kindly provided by Dr. W. L. Alworth, formerly of Tulane University (New Orleans, LA). 7-Ethoxyresorufin and resorufin were purchased from

* To whom correspondence should be addressed. Tel: +81-6-6645-3916. Fax: +81-6-6645-3917. E-mail: shimada@msic.med.osaka-cu.ac.jp.
† Osaka City University Medical School.
‡ Showa Pharmaceutical University.
§ Vanderbilt University School of Medicine.

[1] Abbreviations: EROD, 7-ethoxyresorufin O-deethylation; PAHs, polycyclic aromatic hydrocarbons; 1EP, 1-ethynylpyrene; 1PP, 1-(1-propynyl)-pyrene; 2EP, 2-ethynylpyrene; 4Pbi, 4-(1-propynyl)biphenyl; B[*a*]P, benzo[*a*]pyrene; B[*a*]A, benz[*a*]anthracene; B[*b*]FA, benzo[*b*]fluoranthene; B[*j*]FA, benzo[*j*]fluoranthene; DB[*a,j*]Ac, dibenz[*a,j*]acridine; DMBA, 7,12-dimethylbenz[*a*]anthracene; FA, fluoranthene; 5MeCh, 5-methylchrysene.

10.1021/tx600299p CCC: $37.00
Published on Web 02/10/2007

Scheme 1



Sigma Chemical Co. (St. Louis, MO). B[*a*]P, benz[*a*]anthracene (B[*a*]A), benzo[*b*]fluoranthene (B[*b*]FA), benzo[*j*]fluoranthene (B[*j*]-FA), dibenz[*a*,*j*]acridine (DB[*a*,*j*]Ac), 7,12-dimethylbenz[*a*]anthracene (DMBA), fluoranthene (FA), and 5MeCh (Scheme 1) were obtained from Sigma Chemical Co. and Kanto Kagaku Co. (Tokyo). Other chemicals and reagents used in this study were obtained from sources described previously or were of the highest qualities commercially available (*9*, *12*, *13*).

Enzymes. Bacterial "bicistronic" P450 1A1, 1A2, and 1B1 systems were prepared as described (*9*, *12*). Briefly, plasmids for the expression of P450 1A1, 1A2, or 1B1 plus human NADPH-P450 reductase (using a single promoter) were introduced into *E. coli* DH5α cells by a heat shock procedure, and the transformants were selected in Luria−Bertani medium containing 100 μg ampicillin/mL. Bacterial membranes were prepared and suspended in 10 mM Tris-Cl buffer (pH 7.4) containing 1.0 mM EDTA and 20% glycerol, v/v (*9*, *12*).

Enzyme Assays. Most of the EROD assays were done at 25 °C, unless otherwise stated. Reaction mixtures (2.5 mL) contained recombinant P450 1A1 (12.5−25 pmol), P450 1A2 (100−400

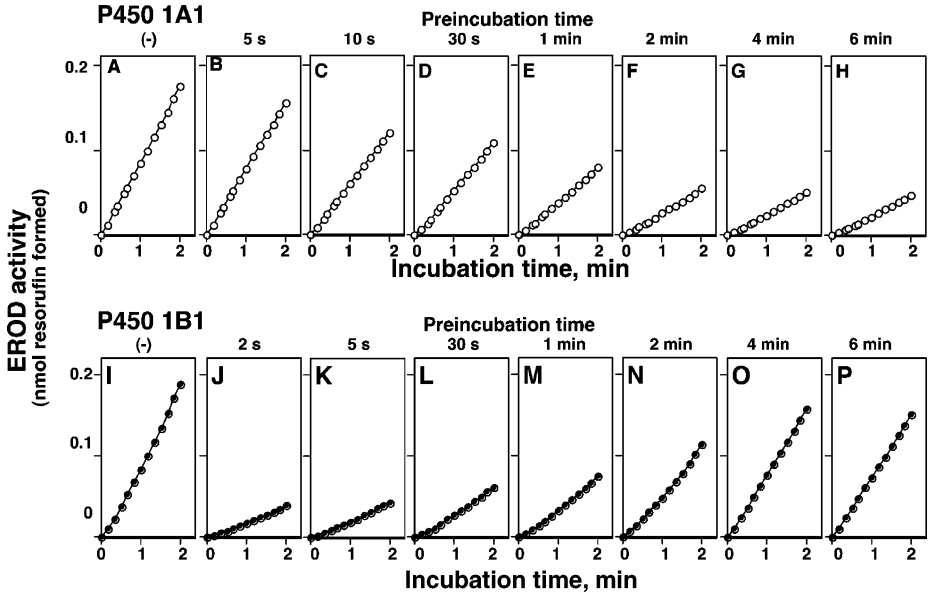


Figure 1. Effects of preincubation time on inhibition of EROD activities by 1PP in systems containing P450 1A1 (parts A−H) and P450 1B1 (parts I−P). The incubation mixture (2.5 mL) contained 12.5 pmol P450 1A1 (parts A−H) or 50 pmol P450 1B1 (parts I−P), 1 mM NADPH, 100 mM potassium phosphate buffer (pH 7.4), and 50 pmol of 1PP (for P450 1A1) or 250 pmol of 1-PP (for P450 1B1). 1PP was not added in parts A (P450 1A1) and I (P450 1B1). 1PP was preincubated with P450 1A1 or 1B1 for varying periods of time (as indicated at the top of each figures), and then reactions were started by adding 5 μM 7-ethoxyresorufin. Reactions were done at 25 °C.

pmol), or P450 1B1 (50−100 pmol), NADPH (2.5 μmol), and 7-ethoxyresorufin (12.5 nmol). Reactions were started by adding either NADPH or 7-ethoxyresorufin, and product (resorufin) formation was monitored directly in a Hitachi F-4500 spectrofluorometer using an excitation wavelength of 571 nm and an emission wavelength of 585 nm (*9*, *14*). All of the PAHs and 7-ethoxyresorufin were dissolved in $(CH_3)_2SO$ and added directly to the incubation mixtures; the final concentration of organic solvent in the mixture was <0.4%.

Metabolism-dependent inhibition of P450s by chemicals was determined as follows. P450s were mixed with 0.10 M potassium phosphate buffer (pH 7.4) containing chemical inhibitors and 7-ethoxyresorufin, and the reaction was started by the addition of NADPH. The increase in resorufin formation was monitored directly for 2−6 min. Metabolism-dependent inhibition of P450 by chemicals was determined using the pseudo-first-order time-dependent losses of EROD activity essentially according to the methods described previously (*15*−*17*). Briefly, semilog plots of the percent relative activity (activities with versus without inhibitors) were determined, and the losses of activities were calculated from initial linear decreases. The $k_{inactivation}$ and K_I values were estimated from double reciprocal plots of log(percent uninhibited activity) vs inhibitor concentration. For the other method of metabolism-dependent inhibition of P450s by chemicals (preincubation method), the inhibitors were first incubated with P450s in the presence of NADPH and then the reaction was started by the addition of 7-ethoxyresorufin.

P450 and protein concentrations were estimated by methods described previously (*18*, *19*). Kinetic parameters for inhibition of EROD activities by PAHs and other compounds were estimated by nonlinear regression analysis using the program KaleidaGraph (Synergy Software, Reading, PA).

Results

Inhibition of P450 1A1-, 1A2-, and 1B1-Dependent EROD Activity by 1PP, 1EP, 2EP, and 4Pbi. We examined inhibition of P450 1A1-, 1A2-, and 1B1-dependent EROD activities by 1PP, 1EP, 2EP, and 4Pbi, which have been shown to be potent inhibitors of human P450 1 family enzymes (*15*, *20*). P450 1A1 or 1B1 was preincubated with 1PP in the presence of NADPH for different periods of time, and then the reaction was started by the addition of 7-ethoxyresorufin (Figure 1). The P450 1A1-dependent EROD activity (without 1PP) was determined to be 6.0 nmol/min/nmol P450, and the activities were decreased with increasing preincubation time when 1PP was present in the incubation mixture (Figure 1A−H). In contrast, P450 1B1-dependent EROD activity (control activities, 1.8 nmol resorufin formed/min/nmol P450) was inhibited directly (without metabolism) by 1PP, and such decreases in activities were reversed with increasing preincubation time (Figure 1I−P).

Effects of preincubation of 1PP, 1EP, 4Pbi, and 2EP with P450 1A1, 1A2, and 1B1 on EROD activities were examined (Figure 2). Preincubation of 1PP, 1EP, and 4Pbi with P450 1A1 caused inhibition of EROD activities in a time-dependent manner (Figures 2A, 2B, and 2C). However, 2EP inhibited P450 1A1 directly (Figure 2J). P450 1B1-dependent EROD activity was inhibited by 1PP and 1EP without metabolism, and such decreases in activities were reversed with increasing preincubation time (Figures 2C and 2F). 4Pbi inhibited P450 1B1 in a mechanism-based manner as in the case of P450 1A1, although such inactivation by P450 1B1 ($t_{1/2}$ = 3.4 min) was slower than that of the P450 1A1 ($t_{1/2}$ = 15 s) (Figures 2I and 2G). 2EP inhibited P450 1B1 directly. All four of the chemicals inhibited P450 1A2 directly.

Metabolism-Dependent Inhibition of P450 1A1 and 1B1 by 1PP, 1EP, and 4Pbi. The above results suggested that 1PP, 1EP, and 4Pbi are mechanism-based inhibitors for P450 1A1; only 4Pbi inhibited P450 1B1 after preincubation. P450 1B1 also reduced the inhibitory potency of 1PP and 1EP as a result of metabolism. To determine the roles of metabolism of 1PP, 1EP, and 4Pbi by P450 1A1 and 1B1, we analyzed the kinetics of inhibition of the P450s (Figures 3 and 4). First, different concentrations of 1EP were mixed with P450 1A1 (Figures 3A and 3C) or P450 1B1 (Figures 3B and 3D) and 7-ethoxyresorufin, and then the reactions were initiated by the addition of NADPH. In both cases, the fits were not linear; the EROD activity of P450 1A1 decreased with incubation time in the presence of 1EP, but the activity of P450 1B1 gradually increased with incubation time. The formation of resorufin was decreased with increasing incubation time when P450 1A1 was incubated with different concentrations of 1EP (Figure 3A). Interestingly, the plots of resorufin formation were more affected by higher concentrations of 1EP with P450 1A1 (traces b, c, d, and e in Figure 3A). Such attenuated EROD rates due to 1EP activation were not seen with P450 1B1; the activities *increased* with incubation time when low concentrations of 1EP were present (Figure 3B). The semilog plots (of percent relative activities) indicated that P450 1A1-dependent EROD activities decreased with incubation time, with the decreases more evident at higher concentrations of 1EP (Figure 3C). In contrast, P450

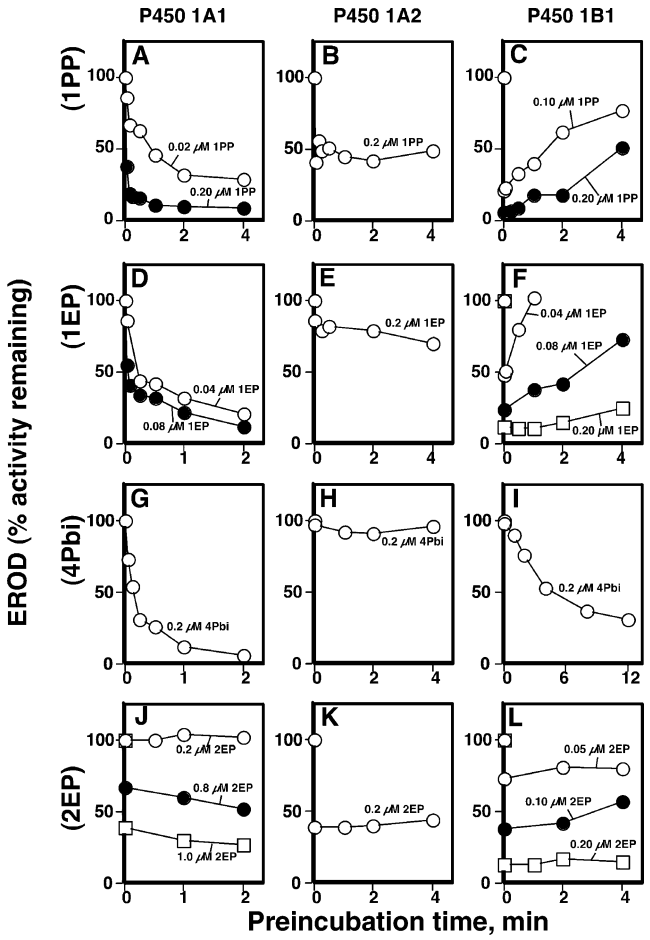


Figure 2. Inhibition of P450 1A1 (parts A, D, G, and E), P450 1A2 (parts B, E, H, and K), and P450 1B1 (parts C, F, I, and L) by 1PP (parts A-C), 1EP (parts D−F), 4Pbi (parts G-I), and 2EP (parts J-L). P450 1A1 (12.5 pmol), P450 1A2 (100 pmol), or P450 1B1 (50 pmol) was preincubated with different concentrations of 1PP, 1EP, 2EP, or 4Pbi in the presence of 1 mM NADPH during the indicated periods of time, and then the reactions were started by the addition of 5 μM 7-ethoxyresorufin. The reactions were monitored for 2 min at 25 °C. The control activities (nmol resorufin formed/min/nmol P450) in the absence of inhibitors were 5.6 ± 0.5 for P450 1A1, 0.85 ± 0.1 for P450 1A2, and 1.7 ± 0.2 for P450 1A2.

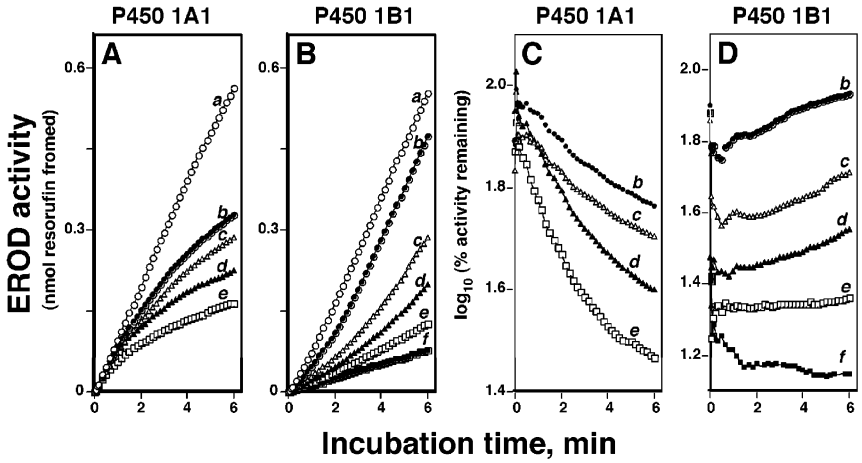


Figure 3. Effects of 1EP on EROD activities catalyzed by P450 1A1 (parts A, C) and P450 1B1 (parts B, D). Reaction mixtures contained P450 1A1 (12.5 pmol) or P450 1B1 (50 pmol), 1 mM NADPH, 100 mM potassium phosphate buffer (pH 7.4), different concentrations of 1-EP [dissolved in $(CH_3)_2SO$], and 5 μM 7-ethoxyresorufin. Reactions were started by adding 1 mM NADPH at 25 °C. The 1-EP concentrations used were 0 (a), 25 nM (b, tan), 50 nM (c, blue), 100 nM (d, red), and 200 nM (e, green) for P450 1A1 (parts A, C), and 0 nM (a), 25 nM (b, tan), 38 nM (c, blue), 50 nM (d, red), 100 nM (e, green), and 200 nM (f) for P450 1B1 (parts B, D). In parts A and C, the formation of resorufin was monitored fluorometrically (for P450 1A1 and P450 1B1, respectively). In parts B and D, EROD activities are presented as log(% activity remaining), as described in Experimental Procedures.

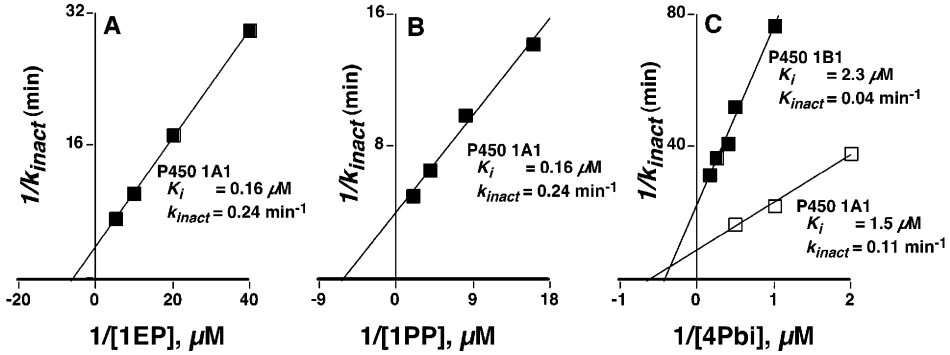


Figure 4. Mechanism-based inhibition of P450 1A1 by 1PP (A), 1EP (B), and 4Pbi (C), and of P450 1B1 by 4Pbi (C). P450 1A1 (12.5 pmol) or P450 1B1 (50 pmol) was incubated with different concentrations of inhibitors, and the formation of resorufin was monitored at 25 °C.

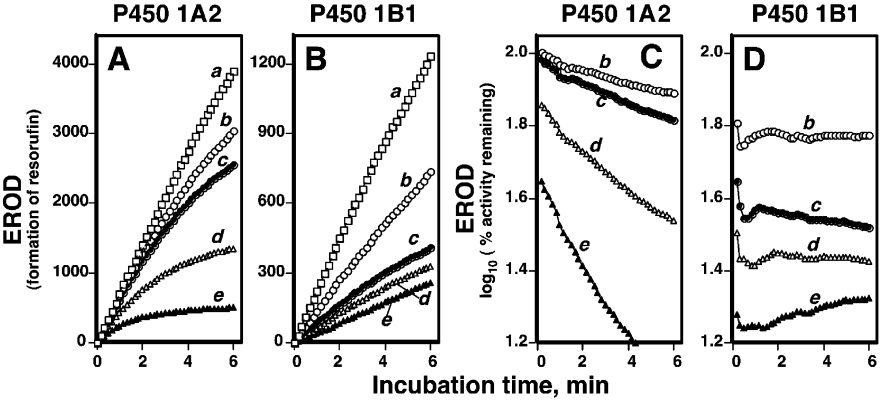


Figure 5. Effects of B[*a*]P on EROD activities catalyzed by P450 1A2 (parts A, C) and P450 1B1 (parts B, D). Reaction mixtures contained P450 1A1 (12.5 pmol) or P450 1B1 (50 pmol), 1 mM NADPH, 100 mM potassium phosphate buffer (pH 7.4), different concentrations of B[*a*]P, and 5 μM 7-ethoxyresorufin. Reactions were started at 25 °C by the addition of 1 mM NADPH. B[*a*]P concentrations used were 0 (a), 0.25 μM (b), 0.5 μM (c), 1.0 μM (d), and 2.0 μM (e) for P450 1A1 (A, C), and 0 (a), 2.5 nM (b), 5.0 nM (c), 10 nM (d), and 20 nM (e) for P450 1B1 (B, D). In parts A and C, the formation of resorufin was monitored fluorometrically for P450 1A1 and P450 1B1, respectively. In parts B and D, EROD activities were presented as log(% activity remaining), as described in the Experimental Procedures.

1B1-dependent EROD activities first decreased and then increased with incubation time (Figure 3D), except at the higher 1EP concentrations where EROD activities were highly inhibited. It is not known at present whether the observed inhibition of P450 1B1 activities at higher concentrations of 1EP is due to the remaining chemical that is not oxidized by the action of P450 1B1.

Kitz−Wilson plots of the metabolism-dependent inhibition of P450 1A1 by 1EP yielded K_i and $k_{inactivation}$ values of 0.16 μM and 0.24 min^{-1}, respectively (Figure 4A). Similar values were obtained with 1PP (Figure 4B) and less efficient rates with 4Pbi (K_i = 1.5 μM and $k_{inactivation}$ = 0.11 min^{-1}) (Figure 4C). The K_i and $k_{inactivation}$ values for the inactivation of P450 1B1 by 4Pbi were 2.3 μM and 0.04 min^{-1}, respectively (Figure 4C).

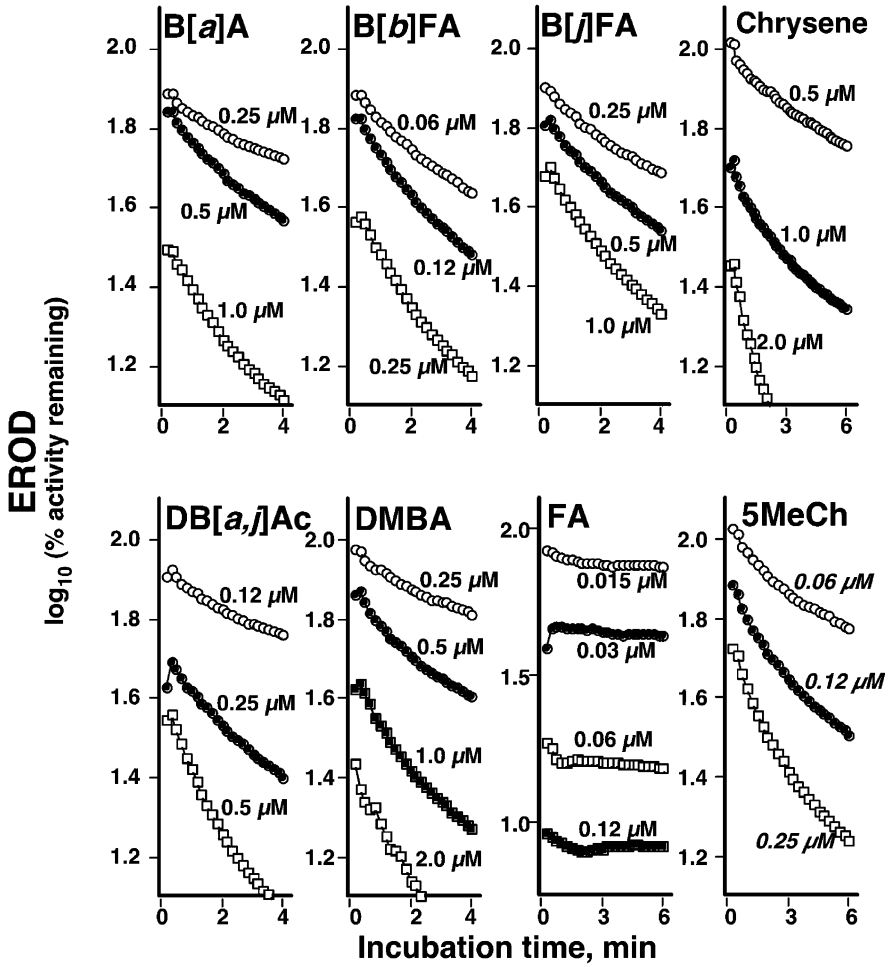


Figure 6. Metabolism-dependent inhibition of P450 1A2-dependent EROD activities by B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB[*a*,*j*]Ac, DMBA, FA, and 5MeCh. Reaction mixtures contained P450 1A2 (100 pmol), 100 mM potassium phosphate buffer (pH 7.4), different concentrations of PAHs (dissolved in DMSO), and 5 μM 7-ethoxyresorufin. Reactions were started by adding 1 mM NADPH and the formation of resorufin was monitored fluorimetrically at 25 °C.

Inhibition of P450 1A1, 1A2, and 1B1 by PAHs. P450 1A2 and 1B1 were mixed with 7-ethoxyresorufin in the presence or absence of B[*a*]P, and reactions were started by the addition of NADPH (Figure 5). EROD activities with P450 1A2 were decreased with incubation time when different concentrations of B[*a*]P were present between 0.25 μM and 2.0 μM (Figure 5A). Surprisingly, semilog plots of relative activities indicated that B[*a*]P inhibited P450 1A2-dependent EROD activities in a metabolism-dependent manner (Figure 5C). In contrast, B[*a*]P inhibited P450 1B1 directly at concentrations between 2.5 and 20 nM (Figure 5B); no changes were seen with time (Figure 5D). P450 1A1 was also found to be directly inhibited by B[*a*]P (results not shown).

Other PAH compounds (including B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB[*a*,*j*]Ac, DMBA, FA, and 5MeCh) were also examined as to whether these chemicals inhibit P450 1A2 in a metabolism-dependent manner (Figure 6). The semilog plots of relative activities showed that these PAHs, with the exception of FA, appeared to be mechanism-based inhibitors of P450 1A2. FA (at concentrations of 0.015, 0.031, 0.062, and 0.125 μM) directly inhibited P450 1A2 EROD.

P450 1A1 and 1B1 were incubated with various PAHs, and semilog plots of EROD activities were examined (Figure 7). The results with B[*a*]P, B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB-[*a*,*j*]Ac, DMBA, FA, and 5MeCh (at single or multiple concentrations) suggested that these PAHs inhibited P450 1A1 directly (upper panels of Figure 7). Similarly, P450 1B1 was directly inhibited by B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB-[*a*,*j*]Ac, DMBA, FA, and 5MeCh without metabolism (lower panel in Figure 7).

The roles of metabolism of PAH inhibitors in the inhibition of P450 1A2 were also examined using a preincubation method (Figure 8). As in the results shown in Figure 6, B[*a*]P, B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB[*a*,*j*]Ac, DMBA, and 5MeCh inhibited P450 1A2-dependent EROD activity following metabolism of these PAHs by P450 1A2. FA was again found to inhibit P450 1A2 directly (Figure 8).

We also determined the effects of metabolism of PAHs on the inhibition of P450 1A1- and 1B1-dependent EROD activities (with the preincubation method) and again found that these PAHs inhibited P450 1A1 and 1B1 directly (results not shown).

Discussion

We recently reported that various PAH compounds strongly inhibit human P450 1A1, 1A2, and 1B1, which have been shown to be the major enzymes involved in the metabolism of these compounds (*1*, *4*, *9*). These results are of interest because humans are exposed to various types of PAH compounds through the environment, and it is assumed that one of the compounds may modify the biological activities of other compounds by changing the metabolism by P450 and other enzymes (*5*−*7*). In order to understand the basis of biological activities of complex mixtures of environmental PAHs in

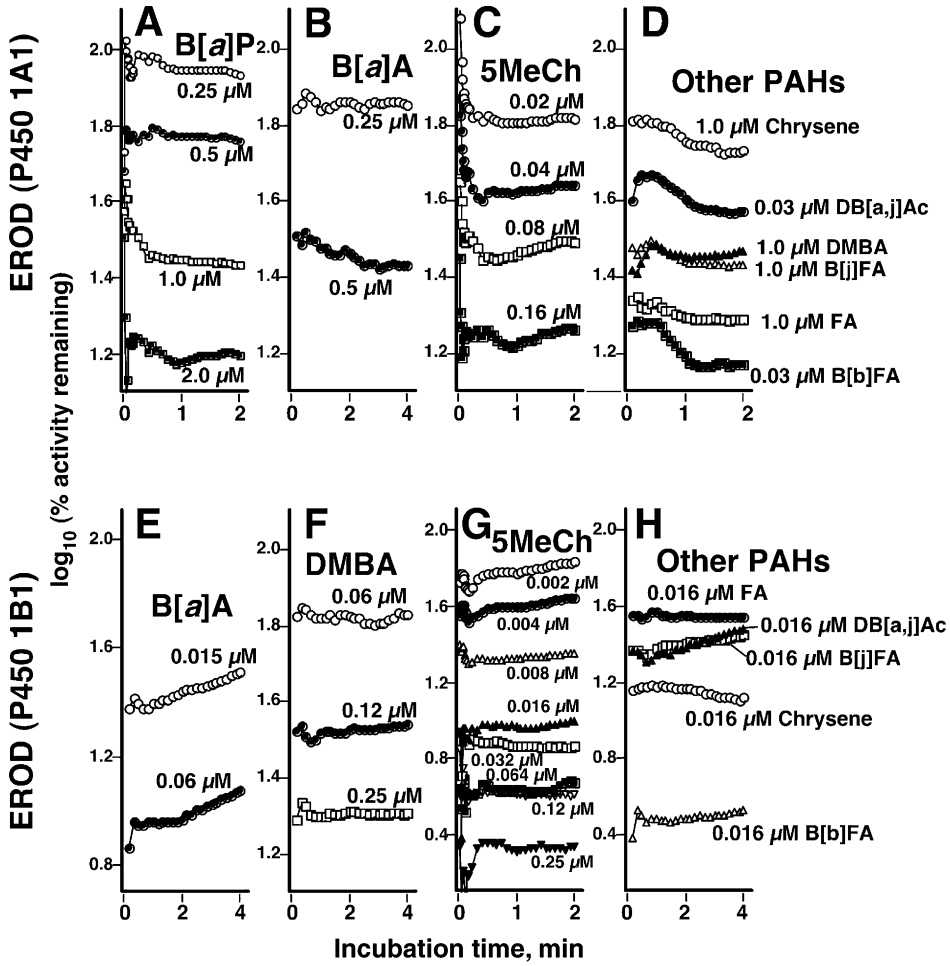


Figure 7. Inhibition of P450 1A1-dependent EROD activities (parts A−D) by B[*a*]P, B[*a*]A, 5MeCh, and other PAHs and of P450 1B1-depedent EROD activities (Parts E−H) by B[*a*]A, DMBA, 5-MeCh, and other PAHs. Reaction mixtures contained P450 1A1 (12.5 pmol) or P450 1B1 (50 pmol), 100 mM potassium phosphate buffer (pH 7.4), different concentrations of PAHs and 5 μM 7-ethoxyresorufin. Reactions were started at 25 °C by the addition of 1 mM NADPH, and the formation of resorufin was monitored fluorimetrically. The PAH concentrations used are described in the figure.

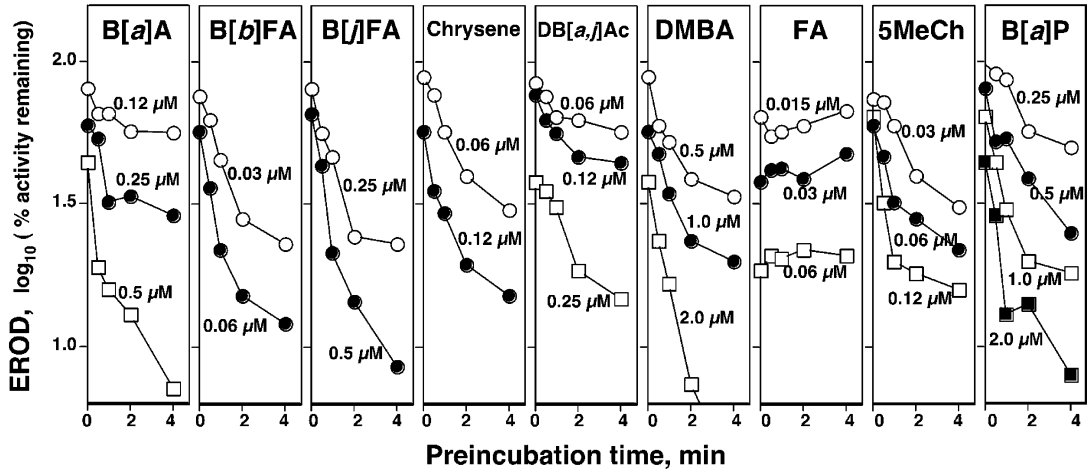


Figure 8. Effects of preincubation time on inhibition of P450 1A2-dependent EROD activities by B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB[*a,j*]Ac, DMBA, FA, 5MeCh, and B[*a*]P. P450 1A2 (200 pmol) was preincubated for the indicated periods of time with different concentrations of PAHs, and then 7-ethoxyresorufin was added to determine EROD activities at 25 °C. The concentrations of PAHs used are indicated in the panels.

humans, it is necessary to determine the mechanisms of these PAH on inhibition of human P450 1A1, 1A2, and 1B1. In this study, we first examined four synthetic model compounds, 1PP, 1EP, 2EP, and 4Pbi, which have been reported to be potent inhibitors of P450 activities, and then studied PAH compounds, most of which are carcinogens in rodents (*1*, *9*, *15*).

The present results indicate that 1PP and 1EP are metabolically activated by P450 1A1 to products that inhibit the enzyme itself and that both compounds inhibit P450 1B1 directly. Interestingly, P450 1B1 was also found to oxidize 1PP and 1EP to products that are less inhibitory to the enzyme. Thus, 1PP and 1EP interact with P450 1A1 and 1B1 differently, and these

Table 1. Summary of Mechanism-Based Inhibition of P450 1A1, 1A2, and 1B1 by PAHs and Arylacetylenes

	P450 1A1			P450 1A2			P450 1B1		
PAH	mechanism-based	K_i (μM)	$k_{inactivation}$ (min^{-1})	mechanism-based	K_i (μM)	$k_{inactivation}$ (min^{-1})	mechanism-based	K_i (μM)	$k_{inactivation}$ (min^{-1})
1EP	(+)	0.16	0.24	(−)	ND[a]	ND	(−)	ND	ND
1PP	(+)	0.16	0.24	(−)	ND	ND	(−)	ND	ND
2EP	(−)	ND	ND	(−)	ND	ND	(−)	ND	ND
4Pbi	*(+)*	*1.5*	*0.11*	*(−)*	*ND*	*ND*	*(+)*	*2.3*	*0.04*
*B[a]*A	*(−)*	*ND*	*ND*	*(+)*	*2.6*	*0.50*	*(−)*	*ND*	*ND*
*B[b]*FA	*(−)*	*ND*	*ND*	*(+)*	*0.11*	*0.20*	*(−)*	*ND*	*ND*
*B[j]*FA	*(−)*	*ND*	*ND*	*(+)*	*0.38*	*0.15*	*(−)*	*ND*	*ND*
*B[a]*P	(−)	ND	ND	(+)	8.3	0.63	(−)	ND	ND
chrysen e	*(−)*	*ND*	*ND*	*(+)*	*13*	*1.1*	*(−)*	*ND*	*ND*
DB[a,j] Ac	(−)	ND	ND	(+)	0.95	0.38	(−)	ND	ND
DMBA	(−)	ND	ND	(+)	1.5	0.30	(−)	ND	ND
FA	(−)	ND	ND	(−)	ND	ND	(−)	ND	ND
5MeCh	(−)	ND	ND	(+)	0.12	0.29	(−)	ND	ND

[a] ND, not determined.

two P450 enzymes produce different types of 1PP and 1EP products. In our preliminary studies, 1EP was found to be transformed by P450 1B1 very rapidly; the chemical disappeared completely from the incubation mixture within 10 min, but 1EP was still present when P450 1B1 was replaced by P450 1A1. One of the products of 1EP by P450 1B1 had a wavelength maximum of 358 nm as well as 224 nm; 1EP itself has a wavelength at 224 nm. The detailed identification of 1EP products formed by P450 1B1, as well as with P450 1A1, is under investigation. Gan et al. (*21*). reported that 1EP is oxidized by liver microsomes (from β-naphthoflavone-treated rats) to fluorescent products, although the structures of the products were not identified. It has previously been proposed that PAH acetylenes and related chemicals are activated by P450 enzymes to reactive intermediates or products that bind covalently to a heme nitrogen or nucleophilic moieties of the enzymes (*17*, *22*, *23*). Further studies are required to examine how 1PP and 1EP inhibit P450 1B1 directly and also which products of 1PP and 1EP attack P450 1A1.

P450 1B1, as well as P450 1A1, appears to activate 4Pbi to a P450 inhibitor (Figure 4). In contrast, 2EP was found to inhibit P450 1A1, 1A2, and 1B1 directly, without activation (Figures 2J, 2K, 2L). Interestingly, the four compounds 1PP, 1EP, 4Pbi, and 2EP all inhibited P450 1A2 directly (Figures 2B, 2E, 2H, 2K). These results suggest different mechanisms of inhibition of P450 1A1, 1A2, and 1B1 by these inhibitors. Interactions between the individual P450 enzymes and inhibitors may determine how these chemicals inhibit P450s and which products react with P450s.

In this study, we used two methods to evaluate inhibition of P450s by chemical inhibitors. In one, P450s were first mixed with substrate and inhibitors and then the reaction was monitored with a spectrofluorometer after adding NADPH. In the other, inhibitors were first preincubated with P450s in the presence of NADPH to generate products and the EROD activities were then monitored, after the addition of 7-ethoxyresorufin (preincubation method). The EROD reaction kinetics could be monitored more exactly, yielding more experimental points (Figures 3 and 4). Double-reciprocal plots of the measured inhibition constants yield parameters for the mechanism-based inhibition of P450s by the chemicals (Figure 5). The potencies of inhibition of P450 1A1 by 1PP and 1EP were similar; the K_i and $k_{inactivation}$ values were 0.16 μM and 0.24 min^{-1}, respectively, in both cases. The mechanism-based inhibition of P450 1A1 by 4Pbi was less efficient than with 1PP and 1EP; the K_i and $k_{inactivation}$ values were 1.5 μM and 0.11 min^{-1}, respectively. P450 1B1 was also found to activate 4Pbi, although the potency was not so great ($K_i = 2.3$ μM, $k_{inactivation} = 0.04$ min^{-1}) compared with P450 1A1.

Various PAH compounds, of which many are carcinogenic but others noncarcinogenic in laboratory animals, have been shown to inhibit strongly P450 1A1, 1A2, and 1B1, the major enzymes involved in activating PAHs to carcinogenic and mutagenic metabolites (*1*, *2*, *12*). The results are of interest because humans are exposed to various types of environmental carcinogens, and each of these complex mixtures may cause either additive or inhibition effects in tumor formation by other PAH compounds (*24*−*28*).

The present results showed that eight of nine PAHs, with the exception of FA, were activated by P450 1A2 to products that inhibit the enzyme and that all nine PAHs inhibited P450 1A1 and 1B1 directly (Figures 6, 8). Numerous studies have shown that B[*a*]P and other PAHs are oxidized by P450s to various products, such as phenols, epoxides, and quinones (*1*, *2*, *4*, *29*, *30*). P450 1A2 has been reported to be generally weaker in activating these PAHs than P450 1A1 and 1A2 (*12*). Several PAH metabolites have been shown to be able to bind covalently to proteins as well as DNA, thus causing cell toxicity and carcinogenesis (*1*, *2*, *29*). This time- and concentration-dependent inhibition of P450 1A2 (Figures 6, 8) appears to be selective, compared to P450 1A1 and 1B1 (Figure 7) and was surprising. Whether a reaction product or an enzyme−substrate reactive intermediate is involved has not been established.

In conclusion, we showed that different mechanisms are involved in the inhibition of P450 1A1, 1A2, and 1B1 by carcinogenic and noncarcinogenic PAH compounds and the model acetylenes 1PP, 1EP, 2EP, and 4Pbi. 1PP and 1EP were found to be activated by P450 1A1 to products that inhibit P450 1A1 itself, while both compounds inhibit directly P450 1A2 and 1B1. Interestingly P450 1B1, but not P450 1A2, is able to metabolize 1PP and 1EP to products that lose inhibitory activity. Several lines of evidence suggest that different mechanisms are involved in inhibition of P450 1A1, 1A2, and 1B1 by various PAH compounds. P450 1A2 was found to be inhibited by B[*a*]P, B[*a*]A, B[*b*]FA, B[*j*]FA, chrysene, DB[*a*,*j*]Ac, DMBA, and 5MeCh—but not by FA—in an apparent mechanism-based manner (Figures 6, 8), but these PAHs (including FA) inhibited P450 1A1 and 1B1 directly (Figure 7). Thus, the PAH compounds may modify the biological activities of other PAH compounds via metabolism by P450s when complex mixtures of PAHs and other chemicals are ingested into the body simultaneously.

Acknowledgment. This work was supported in part by Grants from the Ministry of Education, Science, and Culture of Japan, the Ministry of Health and Welfare of Japan, and by NIH grants R37 CA090426 and P30 ES000267. We thank Y. Nishio and J. Kawawaki for the use of their spectrofluorometer.

Note Added after ASAP Publication. There was an error in the name of a compound in Scheme 1 in the version published ASAP February 10, 2007; the corrected version was published February 21, 2007.

References

(1) Conney, A. H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res. 42*, 4875−4917.

(2) Pelkonen, O., and Nebert, D. W. (1982) Metabolism of polycyclic aromatic hydrocarbons: Etiologic role in carcinogenesis. *Pharmacol. Rev. 34*, 189−222.

(3) Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., Guengerich, F. P., and Sutter, T. R. (1996) Activation of chemically diverse procarcinogens by human cytochrome P450 1B1. *Cancer Res. 56*, 2979−2984.

(4) Shimada, T. (2006) Xenobiotic-metabolizing enzymes involved in activation and inactivation of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metab. Pharmacokinet. 21*, 257−276.

(5) Shimada, T., Iwasaki, M., Martin, M. V., and Guengerich, F. P. (1989) Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by umu gene response in *Salmonella typhimurium* TA1535/pSK1002. *Cancer Res. 49*, 3218−3228.

(6) Guengerich, F. P., Chun, Y.-J., Kim, D., Gillam, E. M. J., and Shimada, T. (2003) Cytochrome P450 1B1: A target for inhibition in anticarcinogenesis strategies. *Mut. Res.* 523-*524*, 173−182.

(7) Chun, Y.-J., Kim, S., Kim, D., Lee, S.-K., and Guengerich, F. P. (2001) A new selective and potent inhibitor of human cytochrome P450 1B1 and its application to antimutagenesis. *Cancer Res. 61*, 8164−8170.

(8) Chun, Y. J., and Kim, S. (2003) Discovery of cytochrome P450 1B1 inhibitors as new promising anti-cancer agents. *Med. Res. Rev. 23*, 657−668.

(9) Shimada, T., and Guengerich, F. P. (2006) Inhibition of human cytochrome P450 1A1-, 1A2-, and 1B1-mediated activation of procarcinogens to genotoxic metabolites by polycyclic aromatic hydrocarbons. *Chem. Res. Toxicol. 19*, 288−294.

(10) Van Duuren, B. L., Katz, C., and Goldschmidt, B. M. (1973) Cocarcinogenic agents in tobacco carcinogenesis. *J. Natl. Cancer Inst. 51*, 703−705.

(11) Slaga, T. J., Jecker, L., Bracken, W. M., and Weeks, C. E. (1979) The effects of weak or non-carcinogenic polycyclic hydrocarbons on 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene skin tumor-initiation. *Cancer Lett. 7*, 51−59.

(12) Shimada, T., Oda, Y., Gillam, E. M. J., Guengerich, F. P., and Inoue, K. (2001) Metabolic activation of polycyclic aromatic hydrocarbons and their dihydrodiol derivatives and other procarcinogens by cytochrome P450 1A1 and 1B1 allelic variants and other human cytochrome P450 enzymes in *Salmonella typhimurium* NM2009. *Drug Metab. Dispos. 29*, 1176−1182.

(13) Shimada, T., Inoue, K., Suzuki, Y., Kawai, T., Azuma, E., Nakajima, T., Shindo, M., kurose, K., Sugie, A., Yamagishi, Y., Fujii-Kuriyama, Y., and Hashimoto, M. (2002) Arylhydrocarbon receptor-dependent induction of liver and lung cytochromes P450 1A1, 1A2, and 1B1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in genetically engineered C57BL/6J mice. *Carcinogenesis 23*, 1199−1207.

(14) Burke, M. D., and Prough, R. A. (1978) Fluorimetric and chromatographic methods for measuring microsomal biphenyl hydroxylation. *Methods Enzymol. 52*, 399−407.

(15) Foroozesh, M., Primrose, G., Guo, Z., Bell, L. C., Guengerich, F. P., and Alworth, W. L. (1997) Propynylaryl acetylenes as mechanism-based inhibitors of cytochrome P450 1A1, 1A2, and 2B1 enzymes. *Chem. Res. Toxicol. 10*, 91−102.

(16) Hopkins, N. E., Foroozesh, M. K., and Alworth, W. L. (1992) Suicide inhibitors of cytochrome P450 1A1 and P450 2B1. *Biochem. Pharmacol. 44*, 787−796.

(17) Roberts, E. S., Hopkins, N. E., Alworth, W. L., and Hollenberg, P. F. (1993) Mechanism-based inactivation of cytochrome P450 2B1 by 2-ethynylnaphthalene: identification of an active-site peptide. *Chem. Res. Toxicol. 6*, 470−479.

(18) Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem. 193*, 265−275.

(19) Omura, T., and Sato, R. (1964) The carbon monoxide-binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem. 23*, 2370−2378.

(20) Shimada, T., Yamazaki, H., Foroozesh, M., Hopkins, N. E., Alworth, W. L., and Guengerich, F. P. (1998) Selectivity of polycyclic inhibitors for human cytochromes P450 1A1, 1A2, and 1B1. *Chem. Res. Toxicol. 11*, 1048−1056.

(21) Gan, L. S. L., Acebo, A. L., and Alworth, W. L. (1984) 1-Ethynylpyrene, a suicide inhibitor of cytochrome P-450 dependent benzo[*a*]pyrene hydroxylase activity in liver microsomes. *Biochemistry 23*, 3827−3836.

(22) Ortiz de Montellano, P. R., Kunze, K. L., Yost, G. S., and Mico, B. A. (1979) Self-catalyzed destruction of cytochrome P-450: covalent binding of ethynyl sterols to prosthetic heme. *Proc. Natl. Acad. Sci. U.S.A. 76*, 746−749.

(23) Roberts, E. S., Pernecky, S. J., Alworth, W. L., and Hollenberg, P. F. (1996) A role for threonine 302 in the mechanism-based inactivation of P450 2B4 by 2-ethynylnaphthalene. *Arch. Biochem. Biophys. 331*, 170−176.

(24) Marston, C. P., Pereira, C., Ferguson, J., Fischer, K., Hedstrom, O., Dashwood, W. M., and Baird, W. M. (2001) Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumor initiation, PAH-DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. *Carcinogenesis 22*, 1077−1086.

(25) Mahadevan, B., Parsons, H., Musafia, T., Sharma, A. K., Amin, S., Pereira, C., and Baird, W. M. (2004) Effect of artificial mixtures of environmental polycyclic aromatic hydrocarbons present in coal tar, urban dust, and diesel exhaust particulates on MCF-7 cells in culture. *Environ. Mol. Mutagen. 44*, 99−107.

(26) DiGiovanni, J., Rymer, J., Slaga, T. J., and Boutwell, R. K. (1982) Anticarcinogenic and cocarcinogenic effects of benzo[*e*]pyrene and dibenz[*a*,*c*]anthracene on skin tumor initiation by polycyclic hydrocarbons. *Carcinogenesis 3*, 371−375.

(27) Rubin, H. (2001) Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates. *Carcinogenesis 22*, 1903−1930.

(28) Rodgman, A., Smith, C. J., and Perfetti, T. A. (2000) The composition of cigarette smoke: a retrospective, with emphasis on polycyclic components. *Hum. Exp. Toxicol. 19*, 573−595.

(29) Gelboin, H. V. (1980) Benzo[*a*]pyrene metabolism, activation, and carcinogenesis: Role and regulation of mixed-function oxidases and related enzymes. *Physiol. Rev. 60*, 1107−1166.

(30) Bauer, E., Guo, Z., Ueng, Y-F., Bell, L. C., and Guengerich, F. P. (1995) Oxidation of benzo[*a*]pyrene by recombinant human cytochrome P450 enzymes. *Chem. Res. Toxicol. 8*, 136−142.

TX600299P