Activation of Chemically Diverse Procarcinogens by Human Cytochrome P-450 1B1

Tsutomu Shimada,2 Carrie L. Hayes, Hiroshi Yamazaki, Shantu Amin, Stephen S. Hecht, F. Peter Guengerich,3 and Thomas R. Sutter3

Department of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, Tennessee 37212 [T. S. F. P. G.]; Department of Environmental Health Sciences, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland 21205 [C. L. H., T. R. S.]; Osaka Prefectural Institute of Public Health, Nakamichi 1-chome, Higashinari-ku, Osaka 537, Japan [H. Y.]; and American Health Foundation, Valhalla, New York 10595 [S. A., S. S. H.]

ABSTRACT

A human cytochrome P-450 (P450) 1B1 cDNA was expressed in Saccharomyces cerevisiae, and the microsomes containing P450 1B1 were used to examine the selectivity of this enzyme in the activation of a variety of environmental carcinogens and mutagens in Salmonella typhimurium TA1535/pSK1002 or NM2009 tester strains, using the SOS response as an end point of DNA damage. We also determined and compared these activities of P450 1B1 with those catalyzed by recombiant human P450s 1A1 and 1A2, which were purified from membranes of Escherichia coli. The carcinogenic chemicals tested included 27 polycyclic aromatic hydrocarbons and their dihydrodiol derivatives, 17 heterocyclic and aryl amines and aminoazo dyes, three mycotoxins, two nitroaromatic hydrocarbons, N-nitrosodimethylamine, vinyl carbamate, and acrylonitrile. Among the three P450 enzymes examined here, P450 1B1 was found to have the highest catalytic activities for the activation of 11,12-dihydroxy-11,12-dihydrobenzo[a]pyrene, 1,2-dihydroxy-1,2-dihydrotolylmethane, (->)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, 11,12-dihydroxy-11,12-dihydrobenzo[a]pyrene, 3,4-dihydroxy-3,4-dihydrobenzo[c]phenanthrene, 3-amino-1,4-dimethyl-5-fluoropyridine(4,3-b)dinol, 2-aminoanthracene, 3-methoxy-4-aminoazobenzene, and 2-nitropyrene. P450 1B1 also catalyzed the activation of 2-amino-3,5-dimethylimidazo(4,5-f)-quinoxaline, 2-amino-3,8-dimethylimidazo(4,5-f)-quinoxaline, 2-amino-6-fluorodinol, 6-aminochromes, and its 1,2-dihydrodiol, (->)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene, 1,2-dihydroxy-1,2-dihydrobenzo[a]pyrene, 1,2-dihydroxy-1,2-dihydro-5,6-dimethylbenzo[a]pyrene, 2,3-dihydroxy-2,3-dihydrofluoranthen, 3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenzo[j]anthracene, and 6-nitrochromes to appreciable extents. However, P450 1B1 did not produce genotoxic products from benzo[a]pyrene, trans-3,4-dihydroxy-3,4-dihydrobenzo[a]anthracene, trans-8,9-dihydroxy-8,9-dihydrobenzo[a]anthracene, 7,12-dimethylbenzo[j]anthracene and its cis-5,6-dihydrodiol, 5-methylchrysene, 11,12-dihydroxy-11,12-dihydromethylcholanthrene, 1,2-dihydroxy-1,2-dihydrotolylmethane, benzo[c]phenanthrene, 2-amino-6-methylpyrido[1,2-a:3',2'-f]imidazole, 2-acetylaminothiophene, benzidine, 2-naphthylamine, aflatoxin B1, aflatoxin G1, stergmatocystin, N-nitrosodimethylamine, vinyl carbamate, or acrylonitrile in this assay system. P450 1B1 is expressed constitutively in extrahepatic organs, including fetal tissue samples, and is highly inducible in various organs by 2,3,7,8-tetrachlorodibenzo-p-dioxin and related compounds in experimental animal models. Thus, activation of carcinogens by P450 1B1 may contribute to human tumors of extrahepatic origin.

INTRODUCTION

Multiple forms of human P450 (1) contribute significantly to the metabolic activation of a number of procarcinogenic chemicals to their proximate reactive species (2). Among human P450 enzymes examined, P450s 1A1, 1A2, 2E1, and 3A4 are generally recognized to be the major forms involved in the activation of most of the carcinogens in human liver and lung microsomes (3). P450 2E1 activates low molecular weight cancer suspects such as vinyl chloride, benzene, and some nitrosamines (3–5). P450 3A4 is a major P450 enzyme in human liver and some extrahepatic tissues and has been reported to have important roles in the activation of a wide range of environmental carcinogens, including carcinogenic mycotoxins, dihydrodiol derivatives of polycyclic aromatic hydrocarbons, 6-aminochrysene, and tris(2,3-dibromopropyl)phosphate (4, 6). P450 1A1 and 1A2 participate principally in the activation of carcinogenic polycyclic aromatic hydrocarbons and heterocyclic and aryl amines (4, 7–9). P450 1A1 can be present in human liver but is expressed predominately in extrahepatic tissues (3, 7). P450 1A2 is expressed primarily in the liver, and constitutive levels of this enzyme are much greater than those of P450 1A1 (7, 10).

Recently, a new P450 family 1 member, P450 1B1, has been identified in rodent species (11–14) and in humans (15). P450 1B1 amino acid sequences among human, rat, and mouse are 80% similar; between subfamilies, P450 1B1 is equally similar, about 40%, to both P450 1A1 and 1A2 (14). The mouse and rat P450 1B1 enzymes have been shown to catalyze the regioselective metabolism of a prototype polycyclic aromatic hydrocarbon carcinogen, DMBA (16, 17). Furthermore, immunoinhibition studies using microsomes prepared from human cells indicate that such regioselectivity may extend to human CYP1B1 (18). Because P450 1B1 is expressed in numerous tissues and is inducible by exposure to dioxins and polycyclic aromatic hydrocarbons (11, 12, 15), it is of interest to determine the role of P450 1B1 in the oxidation and activation of relevant environmental carcinogens.

The present study was designed to examine the role of human P450 1B1 in the activation of a number of procarcinogenic chemicals and to compare these activities with those of human P450 1A1 and 1A2. The results provide evidence that P450 1B1 is a very important enzyme in the activation of a variety of environmental carcinogens and mutagens, including polycyclic aromatic hydrocarbons and aryl amines.

MATERIALS AND METHODS

Chemicals. Benz[a]anthracene-1,2-diol,6 benz[a]anthracene-3,4-diol, benz[a]anthracene-5,6-diol, benz[a]anthracene-8,9-diol, 3-methylcholanthrene,

6 The abbreviations used are: P450, cytochrome P-450; IQ, amino-3-methylimidazo(4,5-f/-quinoxaline; MeIQ, 2-amino-3,8-dimethylimidazo(4,5-f/-quinoxaline; B(a)P, 2-amino-6-methylpyrido[1,2-a:3',2'-f]imidazole; Trp-P-1, 2-amino-6-methylpyrido[1,2-a:3',2'-f]imidazole; Trp-P-2, 2-amino-6-methylpyrido[1,2-a:3',2'-f]imidazole; Trp-P-3, 3-amino-1,4-dimethyl-5-fluoropyridine(4,3-b)dinol; Ppd, 2-amino-1-methyl-6-phenylimidazo[4,5-e]pyridine; DMBA, 7,12-dimethylbenzo[j]anthracene; 3-MeO-AAB, 3-methoxy-4-aminoazobenzene; AFO, aflatoxin B1; AFL, aflatoxin G1. 7 The suffix "diol" is used in the text to designate the prefix "dihydroxydiol-" for individual polycyclic hydrocarbons; all diols are trans unless specifically denoted "cis."
Two fig of poly(A)+ RNA were loaded in each lane except for those of the adrenal, pituitary, uterus, and mammary tissues, which have 0.5 fig poly(A)+ RNA/lane. The exposure diol, and benzo[b]fluoranthene-9,10-diol were synthesized as described (19-27). Chrysene-1,2-diol, dibenzo[a,h]pyrene-1,12-diol, benzo[g]chrysene-11,12-diol, 4-aminobiphenyl, benzidine, and 2-naphthylamine were provided kindly by Dr. F. Kadlubar (National Center for Toxicological Research, Jefferson, AR). Chrysene-1,2-diol, 5-methylchrysene-1,2-diol, 6-methylchrysene-1,2-diol, 6,5-dimethylchrysene-1,2-diol, dibenz[a,h]pyrene-11,12-diol, benzo[g]chrysene-11,12-diol, benzo[c]-phenanthrene-3,4-diol, fluoranthene-2,3-diol, benzo[j]fluoranthene-4,5-diol, and benzo[b]fluoranthene-9,10-diol were synthesized as described (19-27). Vinyl carbamate was a gift of Prof. J. A. Miller (University of Wisconsin, Madison, WI). Other chemicals used were from the same sources as described previously (4).

**Human Tissue Samples.** Poly(A)+ mRNA human tissue blots and poly(A)+ mRNA samples were obtained from Clontech (Palo Alto, CA). All specimens were from normal Caucasians. Samples represent either a single individual or a pool of multiple individuals and were not matched for sex or age. No information was available concerning smoking history or other potential environmental exposures. mRNA (Northern) blot analysis was performed according to the manufacturer’s instructions. Blots were hybridized successively with human cDNA probes for P450 IA1 or IA2 (15, 28) or for β-ACTIN (Clontech) that were labeled with [α-32P]dCTP to a specific activity of 1–2 × 106 cpm/μg DNA, as described previously (15). Nonspecific hybridization was removed by washing each membrane twice in a solution of 1× SSC buffer (0.15 M sodium chloride and 0.015 M sodium citrate, pH 7.0) containing 0.1% SDS (w/v) at 50°C for 60 min. Hybridization signals were visualized by autoradiography.

**Enzyme Preparations.** Human P450 IA1 cDNA clone was introduced into Saccharomyces cerevisiae, and microsomes containing P450 IA1 protein were prepared as described (15). Recombinant human P450 IA1, IA2, and 3A4 proteins were purified to homogeneity from membranes of Escherichia coli in which modified P450 genes had been introduced (29–31). Rabbit liver NADPH-P450 reductase (32) and cytochrome b5 (Ref. 33; used in the P450 3A4 system) were purified as described.

Assays. P450-dependent activation of procarcinogens to reactive metabolites that cause induction of umu gene expression in tester strains Saltonella typhimurium TA1535/spSK1002 and NM2009 was determined in reconstituted P450-containing systems as described previously (4, 5, 34). The former is a yeast microsomal system containing 0.1% SDS (w/v) at 50°C for 60 min. Hybridization signals were visualized by autoradiography.

**RESULTS**

**RNA Expression of P450 IA1, IA2, and IA3 in Normal Human Tissues.** A previous analysis of human P450 IA1 RNA expression revealed that levels of this 5.2-kb transcript were detectable in multiple adult tissue samples, the kidney sample exhibiting the highest apparent signal relative to the other samples tested (15). In this study, we extended these previous results in three ways: (a) analyses of six additional adult tissue samples representing five additional organs and a second adult kidney sample; (b) comparative analyses of the relative expression of P450 IA1 and IA2 in these same tissue samples; and (c) analyses of tissue samples from five fetal organs.

P450 IA1 gene expression was detected in 12 of the 21 adult tissue RNA samples but in none of the 5 fetal tissue RNA samples (Fig. 1). The size of the RNA (2.8 kb) was the same as reported initially (39). The most intense hybridization signals occurred in the prostate and mammary tissues. The larger RNA species (3.3 kb) seen in the liver is the result of cross-hybridization of the P450 IA1 cDNA probe with P450 IA2 RNA (40). The exposure shown here does not distinguish the two separate bands of 2.8 and 3.3 kilobases, representing P450 IA1 and IA2, but in an autoradiogram from a shorter exposure (data not shown), two bands were detected at the appropriate sizes. The liver P450 IA1 mRNA signal (2.8 kb) was about 30% of the intensity of the P450 IA2 mRNA signal (3.3 kb) in this sample. Because a IA1...
Effects of Rabbit NADPH-P450 Reductase on the Activation of Procarcinogens Catalyzed by P450 1B1 in Yeast Microsomes. Yeast microsomes have been shown to contain significant amounts of NADPH-P450 reductase, which is active in the oxidation of several chemicals when reconstituted with mammalian P450 enzymes (43). Activation of 2-aminoanthracene, Trp-P-1, and (−)-benzo[a]pyrene-7,8-diol to genotoxic metabolites by P450 1B1 in yeast microsomes was determined in the S. typhimurium NM2009 strain in the presence or absence of additional NADPH-P450 reductase (Table 1). P450 1B1 (5 pmol) was found to catalyze the activation of these procarcinogens to reactive metabolites that induced umu gene expression in the tester strain. Because the addition of rabbit NADPH-P450 reductase (10 pmol) to the yeast microsomes increased carcinogen activation activities by 1.2-1.5-fold in all cases, subsequent experiments with P450 1B1 in yeast microsomes were conducted in the presence of a 2-fold molar excess of rabbit NADPH-P450 reductase.

Comparison of Catalytic Specificities of P450 1B1, 1A1, and 1A2 in the Activation of Environmental Carcinogens. Among the 27 polycyclic aromatic hydrocarbons and metabolites examined, dibenzo[a]pyrene-11,12-diol, benzo[g]chrysene-11,12-diol, benzo[c]phenanthrene-3,4-diol, (−)-benzo[a]pyrene-7,8-diol, DMBa-3,4-diol, chrysene-1,2-diol, 5-methylchrysene-1,2-diol, and 5,6-dimethylchrysene-1,2-diol were most actively catalyzed by P450 1B1 when S. typhimurium TA1535/pSK1002 was used as a tester strain (Table 2). Catalytic activities of P450 1B1 and 1A1 were very similar for the activation of (−)-benzo[a]pyrene-7,8-diol and 6-aminochrysene-1,2-diol. Only benzo[b]fluoranthene-9,10-diol was activated to a greater extent by P450 1A1 than P450 1B1. P450 1A2 was generally the least effective in the activation of the polycyclic aromatic hydrocarbons examined.

The three human P450 enzymes were also compared with regard to their abilities to catalyze the activation of 16 aryl amines and related carcinogenic chemicals to genotoxic metabolites in the S. typhimurium NM2009 tester strain (Table 3). As reported previously (4), P450 1A2 was highly active in catalyzing several aryl amines, particularly MeIQ, MetaQ, IQ, Glu-P-1, and 2-acetylaminofluorene. Interestingly, P450 1B1 was the most active of the three enzymes in catalyzing the activation of 2-aminoanthracene and 3-MeO-AAB. P450 1B1 had catalytic activity similar to P450 1A2 in the activation of 2-aminofluorene. The activities of P450 1B1 toward Trp-P-1 and 6-aminochrysene were very similar to those of P450 1A1. The catalytic specificities of P450 1B1 were very similar toward several aryl amines, including MeIQ, IQ, and 6-aminochrysene-1,2-diol. P450

Table 1: Effects of rabbit NADPH-P450 reductase on the activation of procarcinogens catalyzed by yeast microsomes containing human P450 1B1

<table>
<thead>
<tr>
<th>Procarcinogen</th>
<th>P450 1B1</th>
<th>P450 1A1</th>
<th>P450 1A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene</td>
<td>&lt;10</td>
<td>130 ± 20</td>
<td>39 ± 29</td>
</tr>
<tr>
<td>9-Hydroxybenzo[a]pyrene</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]pyrene-4,5-diol</td>
<td>87 ± 34</td>
<td>ND²</td>
<td>ND</td>
</tr>
<tr>
<td>(+)-Benzo[a]pyrene-7,8-diol</td>
<td>1260 ± 180</td>
<td>650 ± 77</td>
<td>210 ± 223</td>
</tr>
<tr>
<td>(−)-Benzo[a]pyrene-7,8-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Dibenz(a,h)pyrene-11,12-diol</td>
<td>2560 ± 50</td>
<td>10 ± 10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]anthracene-1,2-diol</td>
<td>42 ± 12</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]anthracene-3,4-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]anthracene-5,6-diol</td>
<td>35 ± 15</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]anthracene-8,9-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>DBMA</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>DBMA-3,4-diol</td>
<td>870 ± 280</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>DBMA-5,6-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[c]phenanthrene</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[c]phenanthrene-3,4-diol</td>
<td>126 ± 40</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Fluoranthene-2,3-diol</td>
<td>55 ± 3</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]fluoranthene-4,5-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene-9,10-diol</td>
<td>140 ± 30</td>
<td>500 ± 42</td>
<td>180 ± 31</td>
</tr>
<tr>
<td>3-Methylyanthracene-11,12-diol</td>
<td>&lt;10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene-5,6-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Chrysene-1,2-diol</td>
<td>250 ± 60</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5-Methylchrysene-1,2-diol</td>
<td>2000 ± 150</td>
<td>260 ± 20</td>
<td>110 ± 10</td>
</tr>
<tr>
<td>6-Methylchrysene-1,2-diol</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5,6-Dimethylchrysene-1,2-diol</td>
<td>220 ± 25</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Benzo[a]chrysene-11,12-diol</td>
<td>575 ± 63</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6-Aminochrysene-1,2-diol</td>
<td>600 ± 140</td>
<td>690 ± 40</td>
<td>880 ± 110</td>
</tr>
</tbody>
</table>

Table 2: Activation of carcinogenic polycyclic aromatic hydrocarbons by human P450 1B1 expressed in yeast and by recombinant P450 1A1 and 1A2 in S. typhimurium TA1535/pSK1002

Table 3: Activation of carcinogenic aryl amines by human P450 1B1 expressed in yeast and by recombinant P450 1A1 and 1A2 in S. typhimurium NM2009

---

Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 1996 American Association for Cancer Research.
1B1 did not catalyze the activation of Glu-P-1, 2-acetylaminofluorene, benzidine, or 2-naphthylamine.

Several other environmental carcinogens were also tested (Table 4); P450 1B1 did not activate AFB1, AFG1, sterigmatocystin, N-nitrosodimethylamine, vinyl carbamate, or acrylonitrile. 2-Nitropyrene and 6-nitrochrysene activations were catalyzed selectively by P450 IBI and P450s 1A1 and 1A2. Among the three human P450 enzymes examined, P450 1B1 had the highest catalytic activities toward several polycyclic aromatic hydrocarbons, including dibenzo[a,l]pyrene-11,12-diol, benzo[g]chrysene-11,12-diol, benzo[c]phenanthrene-3,4-diol, (±)benzo[a]pyrene-7,8-diol, DMBA-3,4-diol, chrysen-1,2-diol, 5-methylchrysene-1,2-diol, and 5,6-dimethylchrysene-1,2-diol, the nitropolycyclic hydrocarbon 2-nitropyrene, and several aryl amines, including 2-aminoanthracene, 3-MeO-AAB, 6-aminochrysene, and the heterocyclic amine Trp-P-1. P450 1B1 was also found to catalyze the activation of other potentially important carcinogens at a considerably rate, including (−)-benzo[a]pyrene-7,8-diol, 2-aminofluorene, MeIQ, IQ, 6-aminochrysene-1,2-diol, and 6-nitrochrysene. In contrast, P450 1B1 did not catalyze activation of Glu-P-1, 2-acetylaminofluorene, benzidine, 2-naphthylamine, AFB1, AFG1, sterigmatocystin, N-nitrosodimethylamine, vinyl carbamate, or acrylonitrile.

We chose to do these initial screening studies in the absence of other enzymes involved in carcinogen metabolism to simplify the system. The S. typhimurium strains have significant levels of (bacterial) N-acetyltransferase. Sulfortransferases and epoxide hydrolase (presumably not present in the yeast microsomes) might facilitate the formation of some reactive products of the polycyclic hydrocarbons and aryl amines [although several of the polycyclic hydrocarbon diol epoxides have not been found to be good substrates for epoxide hydrolases (49, 50)]. The presence of epoxide hydrolase could complicate the results, by detoxication of genotoxic products. Ultimately, studies with several “Phase II” enzymes will be required with P450 1B1 and the lead compounds identified in this study.

It is interesting to note that P450 1B1 was able to catalyze the activation of both polycyclic aromatic hydrocarbons and aryl amines. It has been a general view that carcinogenic polycyclic aromatic hydrocarbons are activated mainly by P450 1A1 and aryl amines mainly by P450 1A2 in experimental animal models and humans (2). Why P450 1B1 activates such a wide range of carcinogenic chemicals is not known, and additional work will be necessary to elucidate the catalytic mechanisms of P450 1B1 at the molecular level. We should emphasize that the studies reported here deal only with the human P450s 1A1, 1A2, and 1B1. At this time, we cannot make exact predictions of the relative contributions of the orthologues of these enzymes in experimental animals, although some studies have shown high activity of rodent P450 1B1 enzymes toward certain polycyclic hydrocarbons (18).

P450 1B1 exhibited remarkable activity for the metabolic activation of dihydrodiols, in numerous cases being the only enzyme of the three

Table 4: Activation of other procarcinogens by human P450 1B1 expressed in yeast and by recombinant P450 1A1 and 1A2 in S. typhimurium TA1535/pSK1002

<table>
<thead>
<tr>
<th>Procarcinogen</th>
<th>P450 1B1</th>
<th>P450 1A1</th>
<th>P450 1A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Nitropyrene</td>
<td>610 ± 40</td>
<td>110 ± 12</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6-Nitrochrysene</td>
<td>110 ± 18</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
<tr>
<td>AFB1</td>
<td>&lt;10</td>
<td>45 ± 31</td>
<td>150 ± 19</td>
</tr>
<tr>
<td>AFG1</td>
<td>&lt;10</td>
<td>11 ± 10</td>
<td>24 ± 11</td>
</tr>
<tr>
<td>Sterigmatocystin</td>
<td>&lt;10</td>
<td>72 ± 19</td>
<td>&lt;10</td>
</tr>
<tr>
<td>N-Nitrosodimethylamine</td>
<td>&lt;10</td>
<td>ND²</td>
<td>ND</td>
</tr>
<tr>
<td>Vinyl carbamate</td>
<td>&lt;10</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Acrylonitrile</td>
<td>&lt;10</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* ND, not done.

DISCUSSION

P450s 1A1 and 1A2 are well-characterized enzymes that are known to metabolize many commonly occurring carcinogens (3, 29, 44). Because the predicted human P450 1B1 protein is similar to both of these enzymes (15), it was of interest to compare the expression and activity of the P450 family 1 members.

The human tissue RNA blot (Fig. 1) demonstrates the organ-specific distribution of this P450 gene family. Although P450 1A1 and 1A2 is detected only in the liver, P450 1A1 and 1B1 are expressed widely and found in many of the same tissues. Although qualitative, the relative expression of P450 1B1 appears greatest in adult kidney, prostate, uterus, and mammary tissue. Further, recent studies in a variety of human cells of epithelial origin indicate that, like P450 1A1, levels of P450 1B1 are highly inducible in response to Ah receptor agonists, including polycyclic aromatic hydrocarbons, dioxins and related compounds, and certain dietary indole carbinols. In addition, P450 1B1 appears to be the predominant family 1 P450 expressed in fetal tissues (Fig. 1). Current knowledge of fetal P450 content and activities is much less than that of adult tissues. However, several groups have demonstrated the presence of aryl hydrocarbon hydroxylation activity in fetal tissues, including the kidney (45), and this activity has been distinguished from P450 1A1 (46–48), indicating that additional studies of fetal expression of P450 1B1 are warranted.

Using expressed P450 1A1, 1A2, and 1B1 proteins and the S. typhimurium umu gene expression assay, we determined the relative capacity of each family 1 P450 to activate 51 procarcinogenic chemicals. A caveat is that this comparison can only be considered semi-quantitative because of the differences in the expression systems, i.e., yeast microsomes for P450 1B1 versus bacterial expression and reconstitution for P450s 1A1 and 1A2. Among the three human P450 enzymes examined, P450 1B1 had the highest catalytic activities toward several polycyclic aromatic hydrocarbons, including dibenzo[a,l]pyrene-11,12-diol, benzo[g]chrysene-11,12-diol, benzo[c]phenanthrene-3,4-diol, (±)benzo[a]pyrene-7,8-diol, DMBA-3,4-diol, chrysen-1,2-diol, 5-methylchrysene-1,2-diol, and 5,6-dimethylchrysene-1,2-diol, the nitropolycyclic hydrocarbon 2-nitropyrene, and several aryl amines, including 2-aminoanthracene, 3-MeO-AAB, 6-aminochrysene, and the heterocyclic amine Trp-P-1. P450 1B1 was also found to catalyze the activation of other potentially important carcinogens at a considerably rate, including (−)-benzo[a]pyrene-7,8-diol, 2-aminofluorene, MeIQ, IQ, 6-aminochrysene-1,2-diol, and 6-nitrochrysene. In contrast, P450 1B1 did not catalyze activation of Glu-P-1, 2-acetylaminofluorene, benzidine, 2-naphthylamine, AFB1, AFG1, sterigmatocystin, N-nitrosodimethylamine, vinyl carbamate, or acrylonitrile.

Fig. 2. Dose-response curves for the activation of 5-methylchrysene-1,2-diol by recombinant P450 1B1 (•), P450 1A1 (○), P450 1A2 (A), and P450 3A4 (Δ). Experimental details are described in "Materials and Methods."
to activate the test compound (Table 1). This was especially notable for the dihydrodiols in which the epoxide ring would be formed in a sterically hindered bay region or fjord region. Examples include 5-methylchrysene-1,2-diol, 5,6-dimethylchrysene-1,2-diol, dibenz[a,j]pyrene-11,12-diol, benzo[c]phenanthrene-3,4-diol, and benzo[g]chrysene-11,12-diol. The resulting diol epoxides are potent carcinogens in several tumor models, including mouse lung and rat mammary gland (22, 51–55).

5-Methylchrysene, one of the components of tobacco smoke condensate, is a strong carcinogen in experimental animals, whereas 6-methylchrysene is a weak carcinogen (49, 56–59). Although the three human P450 enzymes examined (in the absence of epoxide hydrolase) would have catalyzed activation of 5-methylchrysene to dihydrodiols too slowly to utilize assays directly with this parent compound, P450 1B1 showed the highest activation of the major proximate carcinogen, 5-methylchrysene-1,2-diol, but not of 6-methylchrysene-1,2-diol. These results are of interest, because 5-methylchrysene-1,2-diol, but not of 6-methylchrysene-1,2-diol, is a strong pulmonary carcinogen in mice, and recent results suggest that P450 1B1 is expressed in various organs including lung (Fig. 1; Refs. 14, 15, 17, and 60).

The activation of the dihydrodiols examined in this study is of particular interest with respect to the high expression of P450 1B1 in human mammary tissue. In several cases—fluoranthene-2,3-diol, benzo[c]phenanthrene-3,4-diol, dibenz[a,j]pyrene-11,12-diol, and benzo[g]chrysene-11,12-diol—the resulting diol epoxides have been shown to be potent mammary carcinogens in the rat (52, 53, 61). Human exposure to some of the parent compounds, particularly fluoranthene and benzo[c]phenanthrene, is extensive, and the stable dihydrodiol metabolites could be formed in the liver and transported to the mammary tissue, where oxidation to the diol epoxides could occur. In fact, the formation of fluoranthene-2,3-diol has already been demonstrated in human liver (62). Alternatively, P450 1B1 may activate such procarcinogens in situ. For such compounds, including certain heterocyclic and aryl amines (Table 2), P450 1B1 represents an extrahepatic route of metabolic activation. Such local activation lessens the need to invoke complex pharmacokinetic schemes for the redistribution of more polar and/or conjugated metabolites, subsequent to metabolic activation in the liver, and may provide important insights into organ-specific cancers.

In conclusion, the present results demonstrate for the first time that P450 1B1 is an important enzyme involved in the activation of a number of environmental carcinogens and should be considered regarding mechanisms of development of human cancers.

REFERENCES

34. Shimada, T., and Nakamura, S. Cytochrome P-450-mediated activation of procar-
40. Quattrilochi, L. C., Okino, S. T., Ponderthi, U. R., and Tukey, R. H. Cloning and
41. Parri, G., Jardine, K., and McBurney, M. W. Multiple CARG boxes in the human
cardiac actin gene promoter required for expression in embryonic cardiac muscle cells
42. Cleveland, D. W., Lopata, A. M., MacDonald, R. J., Cowan, N. J., Rutter, W. J., and
Beaune, P., and Massuy, D. Recombinant yeast in drug metabolism. Toxicology, 82:
46. Pelkonen, O. Differential inhibition of aryl hydrocarbon hydroxylase in human foetal
49. Wood, A. W., Chang, R. L., Levin, W., Ryan, D. E., Thomas, P. E., Mah, H. D.,
Karle, J. M., Yagi, H., Jerina, D. M., and Conney, A. H. Mutagenicity and tumori-
genicity of phenanthrene and chrysene epoxides and diol epoxides. Cancer Res., 39:
50. Gorukara, E. M., Belvedere, G., Robinson, R. C., Deutsch, J., Coon, M. J.,
Guengerich, F. P., and Gelboin, H. V. The effect of epoxide hydratase on benzo-
52. Hecht, S. S., Amin, S., Lin, J. M., Rivenson, A., Kurtzke, C., and El-Bayoumy, K.
Mammary carcinogenicity in female CD rats of a diol epoxide metabolite of fluo-
ranthene, a commonly occurring environmental pollutant. Carcinogenesis (Lond.),
54. Amin, S., Desai, D., Dai, W., Harvey, R. G., and Hecht, S. S. Tumorigenicity in
newborn mice of fjord region and other sterically hindered diol epoxides of benzo-
[a]pyrene, dibenzo[a,j]pyrene (dibenzo[def]j)chrysene, 4H-cyclopenta[def]
55. Levin, W., Chang, R. L., Wood, A. W., Thakker, D. R., Yagi, H., Jerina, D. M., and
Conney, A. H. Tumorigenicity of optical isomers of the diastereomeric bay-region
3,4-diol,1,2-epoxides of benzo[g]phenanthrene in murine tumor models. Cancer Res.,
56. Koehl, W., Amin, S., Hecht, S. S., Yamazaki, H., Tateishi, T., and Guengerich, F. P.
57. Melikian, A. A., Amin, S., Huie, K., Hecht, S. S., and Harvey, R. G. Reactivity with
58. El-Bayoumy, K., Amin, S., and Hecht, S. S. Complementary DNA binding of poly-
60. Heath, S. S., El-Bayoumy, K., Rivenson, A., and Amin, S. Potent mammary carci-
61. Day, B. W., Sahali, Y., Hutchins, D. A., Wildschütte, M., Pastorelli, R., Nguyen,
T. T., Naylor, S., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. Fluoranthene metabolism: human and rat liver microsomes display different stereoselective forma-
Activation of Chemically Diverse Procarcinogens by Human Cytochrome P-450 1B1

Tsutomu Shimada, Carrie L. Hayes, Hiroshi Yamazaki, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/13/2979

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.