Role of Aryl Hydrocarbon Receptor-Mediated Induction of the CYP1 Enzymes in Environmental Toxicity and Cancer

Daniel W. Nebert*, Timothy P. Dalton, Allan B. Okey,1 and Frank J. Gonzalez2

Department of Environmental Health and Center for Environmental Genetics (CEG)
University of Cincinnati Medical Center, Cincinnati OH 45267-0056

1Department of Pharmacology, University of Toronto
Toronto, Ontario, Canada M5S 1A8

2Laboratory of Metabolism, National Cancer Institute,
National Institutes of Health, Bethesda, Maryland 20814

*Address correspondence to: Daniel W. Nebert, MD, Department of Environmental Health, University of Cincinnati Medical Center, P.O. Box 670056, Cincinnati OH 45267-0056, U.S.A. Tel. 513-558-4347; Fax 513-558-3562; email dan.nebert@uc.edu

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(Abstract)

The mammalian CYP1A1, CYP1A2 and CYP1B1 genes—encoding cytochromes P450 1A1, 1A2 and 1B1, respectively—are regulated by the aromatic hydrocarbon receptor (AHR). The CYP1 enzymes are responsible for both metabolically activating and detoxifying numerous polycyclic aromatic hydrocarbons (PAHs) and aromatic amines present in combustion products. Many substrates for CYP1 enzymes are AHR ligands. Differences in AHR affinity between inbred mouse strains reflect variations in CYP1 inducibility and clearly have been shown to be associated with differences in risk of toxicity or cancer caused by PAHs and arylamines. Variability in the human AHR affinity exists, but differences in human risk of toxicity or cancer related to AHR activation remain unproven. Mouse lines having one or another of the Cyp1 genes disrupted have shown paradoxical effects: in the test tube or in cell culture these enzymes show metabolic activation of PAHs or arylamines, whereas in the intact animal these enzymes are sometimes more important in the role of detoxication than metabolic potentiation. These data contradict pharmaceutical company policies that routinely test drugs under development: if a candidate drug shows CYP1 inducibility, further testing is generally discontinued—for fear of possible toxic or carcinogenic effects. In the future, use of “humanized” mouse lines, containing a human AHR or CYP1 allele in place of the orthologous mouse gene, is one likely approach to show that the AHR and the CYP1 enzymes in human behave similarly to that in mouse.
Classical cancer studies in the 1930s showed that coal tar applied to a rabbit’s ear causes papillomas, followed by tumors. The active ingredients in coal tar were determined to be polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP). The parent PAH at first was thought to be the carcinogen, and an enzyme that metabolized PAHs thought to be beneficial in detoxication (1). It was subsequently shown that PAHs, metabolized to reactive intermediates, bind covalently to nucleic acids and proteins to form adducts (2); thus, the concept of “metabolic activation” by PAH-metabolizing enzymes was born.

Mammalian cell cultures were found to have BaP hydroxylase (aryl hydrocarbon hydroxylase) activity that becomes highly induced within 12–24 hours upon exposure to various PAHs (3). This paradigm for studying the response of cultured cells to PAH treatment has led to a wealth of knowledge concerning transcription, translation, and signal transduction pathways (4–6).

Some inbred mouse strains are “sensitive” to the PAH inducer, while others are not (7). Breeding sensitive C57BL/6 (B6) with resistant DBA/2 (D2) mice revealed that resistance was inherited largely as an autosomal recessive trait (8). When 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD; dioxin) was realized to be at least 30,000 times more potent than PAHs as an inducer of BaP hydroxylase, B6 and D2 mice were treated with dioxin and the effective dose for 50% induction (ED₅₀) was shifted ~15-fold to the right in the resistant D2, compared to the sensitive B6 mouse (Fig. 1). These data—proven, years later, when the genes were cloned—suggested that the Cyp1a1 gene, which encodes BaP hydroxylase, has an identical amino-acid sequence in both B6 and D2 mice; however, the Ahr gene, which encodes the AHR that regulates CYP1A1 induction, has amino-acid differences responsible for high-affinity (in B6) and poor-affinity (in D2) receptor that binds dioxin or PAHs (reviewed in Refs. 10, 11).
Mice having the high-affinity AHR (and therefore higher CYP1 levels in response to lower doses of PAHs) were subsequently shown to be more prone than mice having the poor-affinity AHR to PAH-induced cancers, mutagenesis, birth defects, uroporphyria, and toxicity of the liver, eye and ovary when the administered PAH is in direct contact with the target organ (4). In contrast, mice having the poor-affinity AHR were at greater risk than high-affinity-AHR mice to developing malignancy or toxicity—such as immunosuppression, immune-system malignancy, or toxic chemical depression of the bone marrow—when the target organ is distant from the incoming PAH. This seeming paradox can be explained by PAH pharmacokinetics, called “first-pass elimination” kinetics (reviewed in Ref. 4).

**PAH-induced CYP1 and cancer studies in humans**

Following reports of the mouse high-affinity/low-affinity AHR paradigm and relationship of high-CYP1 inducibility and cancer (12), a CYP1-inducibility assay was developed in mitogen-activated PAH-treated human lymphocytes (which are transformed 55 h later into lymphoblasts) in culture (13), and an association was shown between high-CYP1-inducibility and bronchogenic carcinoma (14). Following improvements in the assay (15), many labs have found that the distribution of CYP1 inducibility was generally skewed to the left (Fig. 2), i.e. more individuals displayed low, and fewer showed high, CYP1 inducibility. Studying cigarette smokers, more than a dozen laboratories independently found correlations between the high-CYP1-inducibility phenotype and cancer of the lung, larynx or oral cavity (tissues in direct contact with cigarette smoke), compared with no correlation between the high-inducibility phenotype and cancer of the renal pelvis, ureter, or urinary bladder (tissues distant from incoming cigarette smoke) (reviewed in Ref. 10).

Do differences in AHR affinity, similar to those found in mice, exist in human populations? From $K_d$ values in 115 unrelated subjects, a $>12$-fold variation in affinity of the human AHR was
found (Fig. 3), but no known AHR gene polymorphism explains this variation (17). AHR-mediated induction of CYP1 enzymes can lead to genotoxicity, mutation and tumor initiation (6). The AHR is also associated with tumor promotion (18) and enhanced oxidative stress (19) independent of CYP1 activity. It is thus possible that a “high-affinity-AHR” patient might develop cancer of the oral cavity or lung after only 20 or 40 pack-years of smoking, whereas a “poor-affinity-AHR” individual might never develop cancer even after more than 100 pack-years.

It should be noted that the human CYP1B1 gene discovery was relatively late (20), compared to knowledge about the CYP1A1, CYP1A2 and AHR genes that had been developed over more than two decades. Before the CYP1B1 enzyme activity was characterized, CYP1A1 had been believed to be responsible for virtually all BaP hydroxylase activity. CYP1B1 is now known to share with CYP1A1 the PAH-inducible BaP hydroxylase activity (21).

**CYP1A1**

Basal CYP1A1 expression is negligible. High levels of CYP1A1 mRNA, protein, and enzyme activity are detectable following induction by PAHs—in fact, many of the inducers are in turn metabolized by CYP1A1. Inducible CYP1A1 activity is ubiquitous, located in virtually every tissue of the body—including endothelial cells of blood vessels, epithelial cells of the skin and gastrointestinal tract, fetus and embryo (*reviewed in Ref. 6*). Of 12 mutations in and near the human CYP1A1 gene (22), no variant exhibits differences in BaP hydroxylase activity.

Among Japanese, a mutated Msp I site, 450 bp downstream of the last exon (CYP1A1*2A allele), is associated with increased risk of cigarette smoking-induced lung cancer, especially when combined with the glutathione S-transferase mu (GSTM1*0) null mutation (23). An I462V mutation, often associated with the Msp I mutation, was reported to have increased BaP hydroxylase activity (23); however, two independent studies showed that cDNA-expressed BaP
hydroxylase activity in vitro is not different between the \textit{CYP1A1}*1 wild-type and \textit{CYP1A1}*2A, *2B or *2C allelic products (24, 25). Similar associations (between the \textit{CYP1A1}*2 alleles and lung cancer in cigarette smokers) were found in other laboratories in Japan (26–28) and China (29, 30), but not in Caucasians, African Americans, or Eastern Mediterraneans (10). In Asian populations but not in Caucasian or African populations, the \textit{CYP1A1}*2 allelic series might therefore be in linkage disequilibrium with a different mutation involved in cigarette smoking-induced cancer. The human \textit{CYP1A2} gene might be a candidate, because it is located only 24.5 kb from the \textit{CYP1A1} gene (31).

Dozens of other clinical studies of the \textit{CYP1A1} polymorphism and various types of toxicity or cancer have also been reported.

\textbf{CYP1A2}

\textit{CYP1A2} metabolizes some drugs, plus many environmental aromatic amines: \textit{N}-heterocyclic amines found in charcoal-grilled food—such as 2-amino-3-methylimidazo[4,5\textit{f}]quinoline (\textit{IQ}) and 2-amino-1-methyl-6-phenylimidazo[4,5\textit{b}]pyridine (\textit{PhIP})—and arylamines such as 2-acetylaminoﬂuorene and 4-aminobiphenyl (\textit{ABP}). Substantial constitutive \textit{CYP1A2} activity occurs in mammalian liver. The human \textit{CYP1A2} gene is PAH-inducible in liver, GI tract, nasal epithelium and brain. Although there are >60-fold differences in hepatic \textit{CYP1A2} between individuals (10), there have been no mutations shown unequivocally to account for the striking inter-individual differences in levels of expression. At least 14 mutations in and near the human \textit{CYP1A2} gene have been described to date, with three showing decreases in enzyme activity and one showing increases in inducibility (22).

\textbf{CYP1B1}

\textit{CYP1B1} metabolizes numerous PAHs, as well as many \textit{N}-heterocyclic amines, arylamines and amino azo dyes, and several other carcinogens (21). Unlike \textit{CYP1A1}, \textit{CYP1B1} often shows
substantial constitutive levels. CYP1B1 expression is high in vascular endothelial cells, breast, prostate, uterus, epithelial lining of the head and neck, various types of tumors, adrenal cortex, and many other tissues. That the highest BaP hydroxylase activity in the first trimester occurs in human adrenal cortex (32) presumably reflects constitutive CYP1B1 expression. Large inter-individual differences in CYP1B1 (and CYP1A1) protein levels have been reported in human lung (33), although what is constitutive and what is inducible CYP1B1 is difficult to distinguish among smokers, non-smokers and ex-smokers. To date, at least 22 mutations in and near the human CYP1B1 gene have been reported, though none have yet been shown via cDNA-expression assays to cause decreased enzyme activity (22). Many of these mutations are associated with an inborn-error-of-metabolism (primary congenital glaucoma)—suggesting that development of the anterior chamber of the eye during embryogenesis might require metabolism of an important endogenous substrate by CYP1B1 (34). CYP1B1 appears to be largely responsible for PAH-induced immunotoxicity (35).

**AHR**

The AHR is a ligand-activated transcription factor that controls several dozen genes (5, 11, 36), including up-regulation of all three CYP1 genes (6). Ligands for the AHR include dioxin, PAHs, polyhalogenated aromatic hydrocarbons, indoles and tryptophan-derived endogenous ligands, and benzoflavones found especially in cruciferous plants (37). The AHR gene exists in all vertebrates, and even in *Caenorhabditis elegans* (11). The AHR participates in cell-cycle control and apoptosis that is cell type- or tissue-specific (6). To date, at least nine mutations in and near the human AHR gene (17, 38, 39; unpublished data), and a staggering 2,213 mutations in and near the mouse Ahr gene spanning ~16 kb from 13 inbred strains (40), have been reported.
The Ahr(-/-) knockout mouse exhibits lowered viability and fertility and defects in liver
development (41–43). The Ahr(-/-) mouse lacks constitutive and inducible CYP1 expression, and is
resistant to TCDD-induced toxicity (44), topical BaP-induced skin tumors (43), and benzene-
induced hemotoxicity (45). The Ahr(-/-) mouse generated in Japan (46) appears to have high
constitutive CYP1A2 levels in liver but not lung.

Paradoxical effects of CYP1A1 and CYP1A2

Historically, the role of CYP1A1 in BaP-induced toxicity was demonstrated in the Hepa-1c1c7
hepatoma cell line. Mouse Hepa-1 cells retain several differentiated liver functions—including
albumin synthesis, commonly lost in culture (4–6). BaP-treated Hepa-1 cells grew only rarely as
resistant variants; such colonies were used to complement the “resistance” phenotype in other
colonies, which led to the discovery of at least three complementation groups (5). These were
ultimately defined as the genes encoding Cyp1a1, Ahr, and the AHR’s dimerization partner, the
AHR nuclear translocator, Arnt. Thus, CYP1A1 activates BaP to become toxic, and the AHR and
ARNT are necessary for Cyp1a1 inducible expression; these experiments showed that CYP1A1 is a
primary determinant for BaP toxicity. Since these experiments were conducted in hepatoma cells, it
was presumed that hepatic CYP1A1 is likely to be responsible for BaP-mediated toxicity in the
intact animal.

Cyp1a1(-/-) (47), Cyp1a2(-/-) (48) and Cyp1b1(-/-) (49) knockout mice are viable and able to
reproduce. Cyp1a2(-/-) mice exhibit increased toxicity from drugs that are predominantly CYP1A2
substrates (48, 50). When Cyp1a1(-/-) mice were given oral BaP (125 mg/kg/day), however, all
Cyp1a1(-/-) mice die within 30 days while Cyp1a1(+/+) mice survived the year-long experiment;
BaP-DNA adducts are unexpectedly much higher in the gastrointestinal tract, liver, spleen and
marrow of Cyp1a1(-/-), and immunotoxicity occurs, compared with that in wild-type mice (51).
BaP pharmacokinetic studies suggested that adducts accumulate to high levels in Cyp1a1(-/-) mice, despite much lower rates of BaP metabolism in the genetic absence of CYP1A1. The Cyp1a2(-/-) mouse also shows paradoxical responses. Metabolic activation of the human urinary bladder carcinogen ABP by CYP1A2 in vitro causes enhanced ABP-DNA adducts and toxicity, yet Cyp1a2(-/-) mice treated with topical ABP show increased adducts in the liver and urinary bladder (52) (metabolism in the absence of CYP1A2). A similar contradiction was seen in ABP-induced hepatocellular carcinomas and preneoplastic foci (53) and ABP-induced methemoglobinemia (54). Further paradoxical responses were observed with the food mutagens IQ and PhIP on DNA adducts in liver, kidney, mammary gland and colon (55) and the effect of PhIP on the incidence of several types of malignancies (56).

To our knowledge, the Cyp1b1(-/-) mouse has not shown any such inconsistent effects. As might be predicted from in vitro studies, the Cyp1b1(-/-) mouse exhibits increased protection against 7,12-dimethylbenzo[a]anthracene (DMBA)-induced lymphomas (49), DMBA-induced marrow toxicity and pre-leukemia (57) and dibenzo[a,l]pyrene-induced tumors (58). Hence, if CYP1B1 is not present to activate these environmental chemicals, less toxicity or neoplasia is seen.

Thus, in the context of hepatoma cells or in vitro studies, CYP1A1 is the primary determinant of BaP-mediated toxicity and DNA adduct formation and CYP1A2 is the primary determinant of arylamine-mediated toxicity and DNA adduct formation, whereas in the context of the intact animal, CYP1A1 and CYP1A2 can be protective. This dual role has not been seen with CYP1B1. What might explain this difference? In microsomes, 9000 x g supernatant (S9) fractions or Hepa-1 cells, an absence of, or loose coupling to, Phase II metabolizing enzymes (Fig. 4) would result in enhanced adduct formation, oxidative stress and toxicity. In gastrointestinal epithelial cells or hepatocytes, it is possible that CYP1A1 and CYP1A2 are tightly coupled, resulting in efficient
detoxication rather than increases in toxicity. In the genetic absence of CYP1A1 or CYP1A2, other oxidative enzymes—CYP1B1, CYP2 and prostaglandin H synthase for BaP (51); CYP2A and flavin-containing monooxygenases for arylamines (52)—are responsible for adduct formation and toxicity. In immune cells, it is possible that CYP1B1 is not tightly coupled to phase II metabolism, or Phase II metabolism is low or absent, resulting in enhanced BaP-DNA adduct formation and toxicity. An additional likely factor is CYP1 enzyme concentration: gastrointestinal and hepatic CYP1A1 and CYP1A2 are very high in the paradoxical systems described above, whereas CYP1B1 content, in relative terms, is not high in immune cells.

Therefore, in the intact animal the role of CYP1 in detoxication versus activation to cause toxicity is likely to depend on the subcellular content and location, the amount of Phase II metabolism, the degree of coupling to Phase II enzymes, and cell type- and tissue-specific context, as well as pharmacokinetics (route of administration, target organ) of the chemical under study. The notion that CYP1A1 is causative in PAH-mediated toxicity and carcinogenesis (or CYP1A2 causative in ABP-, IQ- or PhIP-mediated toxicity and malignancy) may not be warranted and, in fact, the contrary may be true. These findings underscore the difficulties in using data collected in vitro to extrapolate to the in vivo situation. In vitro data have been invaluable in helping determine the catalytic specificities of CYP1 enzymes; from this perspective, there can be little doubt that CYP1B1 and CYP1A1 represent major cellular activities toward PAH metabolism or that CYP1A2 carries out arylamine metabolism. Their roles in causing, preventing, or not participating in PAH- or arylamine-mediated toxicities, however, need further investigation in the intact animal.

Thus, we have come full-circle. There was a time when CYP1 enzymes were thought to be primarily beneficial because of detoxication (1). Then, we all became convinced that CYP1 enzymes were detrimental in that they caused toxicity and cancer (2, 4, 10, 12, 59). Now, it appears
that, in all likelihood, evolution has provided animals with CYP1 enzymes which, on balance, are
generally more protective than destructive during environmental insult.

**Generation of “humanized” BAC-transgenic mouse lines**

“Humanized” hCYP3A4 and hCYP2D6 mouse lines have been developed, in which these
pharmacologically important human genes were added to the mouse genome; even without the
orthologous mouse Cyp3a and Cyp2d genes removed, these lines have proven very useful for
numerous pharmacological studies (60). A mouse line containing a human AHR cDNA in place of
the mouse Ahr gene has recently been reported (61). Humanized hCYP1A1, hCYP1A2, hCYP1B1
and hAHR mouse lines are now under development, in which the human gene replaces the mouse
orthologous gene. Two or more human genes might also be combined in developing a mouse line.
Such increasingly “humanized” mouse lines will be important in future risk assessment studies of
toxicity and carcinogenesis.

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FIGURE LEGENDS

FIG. 1. Dose-response curve for hepatic CYP1A1 induction by TCDD in B6 and D2 mice. The enzyme activity was determined 3 days after treatment with the indicated doses of TCDD; dosage on the abscissa is logarithmic scale. (Redrawn and modified from Ref. 9, with permission obtained from Elsevier)

FIG. 2. Maximally induced CYP1 enzyme activity, per unit of NADH-cytochrome c reductase activity, in mitogen-activated 3-methylcholanthrene-treated lymphocytes from 47 unrelated individuals. Environmental factors, such as the number of cigarettes smoked at the time the blood was drawn, do not influence this assay which specifically determines the CYP1-inducibility phenotype (Redrawn from Ref. 16, with permission obtained from University of Chicago Press).

FIG. 3. Probit analysis of placenta cytosolic samples from 115 unrelated patients. The dissociation constant, $K_d$, for each patient was determined by Scatchard plot, using five concentrations of radiolabeled $[^3H]$TCDD (Renehan, E., Manchester, D.K., Parker, N.B., Wong, J.M.Y., Giannone, J.V., Bush, L., Endrenyi, L., Harper, P.A., Okey, A.B., unpublished data). A low $K_d$ value by Scatchard analysis would be consistent with the high-affinity B6 curve in Fig. 1 and the high CYP1/reductase ratio in Fig. 2.
FIG. 4. Diagram of Phase I oxidative enzymes and Phase II conjugating enzymes that can be geographically subcellularly “tightly coupled” (top) or “loosely coupled” (bottom). R, any CYP1 substrate. RO⁻, reactive intermediate. RO-Conj, inactive product. Both Phase I enzymes and Phase II enzymes can be membrane-bound, both can be cytosolic, or one can be membrane-bound and the other cytosolic. Phase II metabolism includes glutathione S-transferases, UDP glucuronosyltransferases, and various acetyl-, methyl- and sulfotransferases (6, 10, 21, 59).
NUMBER OF INDIVIDUALS

CYP1 / Reductase activity

N=47
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