

DMD 15826

**CYP1A INDUCTION AND HUMAN RISK ASSESSMENT: AN EVOLVING
TALE OF *IN VITRO* AND *IN VIVO* STUDIES**

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DMD 15826

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Abbreviations used: CYP, cytochrome P450; PAH, polycyclic aromatic hydrocarbons; HAA, heterocyclic aromatic amines/amides; HAH, halogenated aromatic hydrocarbons; B[a]p, benzo[a]pyrene; 3-MC, 3-methylcholanthrene; 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene; AAF, 2-acetylaminofluorene; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; AHH, aryl hydrocarbon hydroxylase; *Ah*, aromatic hydrocarbon responsiveness locus; AhR, aryl hydrocarbon receptor; Arnt, Ah receptor nuclear translocator; DRE, dioxin response element; DDI, drug-drug interaction; bHLH, basic helix-loop-helix; PAS, Per-Arnt-Sim homology domain; EM, extensive metabolizer; PM, poor metabolizer.

DMD 15826

Abstract

CYP1A1 and 1A2 play critical roles in the metabolic activation of carcinogenic polycyclic aromatic hydrocarbons (PAH) and heterocyclic aromatic amines/amides (HAA) to electrophilic reactive intermediates, respectively, leading to toxicity and cancer. CYP1As are highly inducible by PAH and halogenated aromatic hydrocarbons (HAH) via aryl hydrocarbon receptor (AhR)-mediated gene transcription. The impact of CYP1A induction on the carcinogenic and toxic potentials of environmental, occupational, dietary, and therapeutic chemicals has been a central focus of human risk evaluation and has broadly influenced the fields of cancer research, toxicology, pharmacology, and risk assessment over the past half century. From the early discovery of CYP1A induction and its role in protection against chemical carcinogenesis in intact animals, to the establishment of CYP1A enzymes as the principal P450s for bioactivation of PAH and HAA in *in vitro* assays, to the recent realization of an essential protective role of CYP1A in B[a]p-induced lethality and carcinogenesis with CYP1A knockout mice, the understanding of the interrelation between CYP1A induction and chemical safety has followed a full circle. This unique path of CYP1A research underscores the importance of whole animal and human studies in chemical safety evaluation.

DMD 15826

I. Introduction

Humans are constantly exposed to harmful foreign chemicals and materials from dietary, therapeutic, environmental, and occupational sources. As such, defense mechanisms have evolved to protect against toxic insults. The cytochrome P450 family of enzymes plays critical roles in the biotransformation of drugs, carcinogens, steroid hormones, and environmental toxicants (Lu, 1998; Conney, 2003; Guengerich, 2004; Coon, 2005). Cytochrome P450 1A1 and 1A2 catalyze the oxygenation of polycyclic aromatic hydrocarbons (PAH) and heterocyclic aromatic amine/amides (HAA), the demethylation of aminoazo dyes, and the dealkylation of phenacetin and caffeine and other therapeutic agents (Conney, 1982; Kim and Guengerich, 2005). Oxygenation of the chemicals by 1A1 and 1A2 serves as an initial step in the conversion of the substrates to more polar metabolites, resulting in increased excretion and thereby maintaining the chemical homeostasis in the body. However, oxygenation of carcinogenic PAH and HAA (procarcinogen) gives rise to arene oxide, diolepoxide, and other electrophilic reactive species (ultimate carcinogen) that form DNA and protein adducts, leading to tumor formation and toxicity (Gelboin, 1980; Miller and Miller, 1981; Conney, 1982; Jerina, 1983). Humans encounter PAH and HAA from a wide range of sources such as tobacco smoke, automobile exhaust, smoked and cooked food, and industrial processes. Such exposures have been causatively linked to an increased incidence of cancers in certain populations such as smokers (Proctor, 2001). Thus, the metabolic activation of PAH and HAA by P450 1A enzymes is a critical step in the formation of cancer in human populations exposed to PAH and HAA.

DMD 15826

A remarkable feature of P450 1A1/2 action is that the enzymes are highly inducible at both mRNA and enzyme levels by a range of chemicals (Whitlock, 1999; Ma, 2001). Inducers in many cases are substrates of the enzymes such as benzo[a]pyrene (B[a]p) and 3-methylcholentrene (3-MC). CYP1A1 is expressed at low levels in extrahepatic tissues in humans but is highly inducible in the liver and extrahepatic tissues. CYP1A2 is constitutively expressed in the liver and is inducible. Induction of CYP1A by PAH is mediated through the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor. Binding of PAH to AhR elicits sequential signaling events leading to the activation of AhR and transcription of CYP1A genes through the dioxin response element (DRE) located in the enhancers of the genes. In this framework, PAH, AhR, DRE, and CYP1A1/2 form a receptor-mediated transcriptional loop that directly senses the concentrations of PAH in cells and increases the activities of the enzymes through transcription. Induction subsides as PAH is metabolized to ensure that induction is initiated and maintained only as needed. Therefore, induction is not only necessary for clearance of chemicals in the body but also tightly regulated according to the cellular concentrations of xenochemicals.

The biological impact of CYP1A induction can be two-fold. Induction of CYP1A in general serves as a means of maintaining the homeostasis of the chemical environment in cells by increasing the metabolic clearance of substrates. Since CYP1A1/2 catalyzes the metabolic activation of PAH and HAA to ultimate carcinogens, it is expected that induction of the enzymes is detrimental in humans that are exposed to high levels of PAH and HAA such as cigarette smoking. Moreover, induction of the enzymes in humans exhibits large variations (Ma and Lu, 2003); high inducibility may impose additional risk

DMD 15826

for lung cancer to individuals who are smokers. Further, CYP1A1/2 can metabolize a range of substrates; induction of the enzymes by one substrate may increase the metabolism of other chemicals (for instance, clinical drugs), resulting in unexpected drug-drug interactions (DDI).

Because of the critical role of CYP1A in chemical carcinogenesis and toxicity, the implication of CYP1A induction in human risk evaluation has been and remains to be a central focus of interest in cancer research, drug development, toxicology, food safety, and environmental/occupational health since the discovery of CYP1A induction. In this review, we analyzed the evidence accumulated over the past five decades that implicates CYP1A induction in the biological effects of carcinogens, drugs, and environmental chemicals both as beneficial and detrimental responses. It is the authors' hope that such analysis would provide new insights into the human safety analysis of CYP induction and be instrumental to the application of *in vitro* and *in vivo* P450 reactions in human risk assessment.

II. The discovery of CYP1A induction: initial observations of the impact of CYP1A induction on cancer and toxicity

The discovery of CYP1A induction originates from the observation that PAH induces its own metabolism (Conney, 2003). These early studies have also provided the initial *in vivo* evidence demonstrating large impacts of CYP1A induction on the toxicity and carcinogenesis of certain chemicals in animals. In 1952, Richardson and associates (Richardson et al., 1952) reported that co-administration of a strong hepatocarcinogen 3'-methyl-4-dimethylaminoazobenzene (3'-Me-DAB) with a low dose of 3-methylcholanthrene (3-MC) in rats delayed or entirely inhibited liver tumor development

DMD 15826

that was observed in rats treated with 3'-Me-DAB alone. Inhibition was also seen for 3'-Me-DAB-induced liver cirrhosis. The findings demonstrated protection by 3-MC against the carcinogenicity and toxicity of 3'-Me-DAB. Miller et al (Miller et al., 1958) showed that the inhibition of carcinogenesis by 3-MC and other PAHs is not specific for 3'-Me-DAB, but also for tumorigenicity by aminoazo dyes, 2-acetylaminofluorene (AAF), and AAF derivatives.

A critical issue arises from these *in vivo* inhibition studies: what accounts for the protective effect of PAHs? To address the question, Conney et al (Conney et al., 1956) examined the effects of treating rats with 3-MC and other PAHs on the hepatic *N*-demethylation and azo link reduction of aminoazo dyes, which are metabolic pathways resulting in noncarcinogenic products. The findings revealed that PAHs that inhibited aminoazo dye-induced liver cancer were potent inducers of azo dye *N*-demethylase, whereas those PAHs that did not affect azo dye-induced carcinogenesis had little or no effect on azo dye metabolism. This and subsequent other studies provided early evidence of CYP1A induction and a mechanistic explanation for the protective effect of PAHs on azo dye carcinogenesis (Conney, 2003). The studies opened the gateway of research on CYP induction that had profound influence on the development of drug metabolism, cancer research, pharmacology, and toxicology over the following half century.

III. Mouse genetics in CYP1A induction.

Mouse genetics contributed greatly to the understanding of the mechanism of CYP1A induction. Induction was measured as an increase in the aryl hydrocarbon hydroxylase (AHH) activity (i.e. 3-hydroxylation of B[a]p). Some inbred mouse strains such as C57BL/6 (B6), are sensitive to induction by 3-MC; whereas other strains such as

DMD 15826

DBA/2 (D2) are resistant to the induction (Nebert and Gelboin, 1969; Kodama and Bock, 1970). The sensitive phenotype was segregated as a single autosomal dominant trait (Gielen et al., 1972; Thomas et al., 1972). The polymorphism of the genetic trait defines a genetic locus, which was designated as the *Ah* locus (aromatic hydrocarbon responsiveness). *Ah^b* represents the “*Ah* responsive” or B6 allele and *Ah^d* the “*Ah* non-responsive” or D2 allele. Thus, genotypes of *b/b* and *b/d* give high induction of CYP1A, whereas that of *d/d* is non-responsive. Later, the *b* allele was further separated into *b-1* (C57BL/6), *b-2* (C3H), and *b-3* (Mus spretus) alleles (Poland et al., 1994).

Among the inducers of CYP1A, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) was found to be the most potent for the induction of the enzymes. TCDD and 3-MC produce parallel log dose-response curves for the induction of AHH activity. However, TCDD is about 30,000 times as potent as 3-MC (Poland and Glover, 1974). In addition, induction by TCDD is persistent compared with that by PAH (>35 vs 8 days) due to its long half life ($t_{1/2} = 17$ days in rats); the symmetrical localization of chlorine atoms on the ring structure of TCDD gives rise to its resistance to metabolic breakdown and prolonged $t_{1/2}$. Induction by TCDD and its congeners is stereospecific in structure. While TCDD potently induces AHH with an ED_{50} of 1 nmole/kg in responsive strains, the induction is also observed in all non-responsive strains with an ED_{50} of about 10 nmoles/kg (Poland and Glover, 1975). The heterozygous genotype (*b/d*) exhibits intermediate sensitivity to TCDD, which is distinguishable from *b/b* and *d/d* strains (Niwa et al., 1975). These observations suggest a diminished affinity for inducers in D2 mice as a cause of non-responsive phenotype in CYP1A induction by PAHs (Poland and Glover, 1975).

DMD 15826

The comparative studies on the potency and stereospecificity among the inducers and between the *Ah* responsive and non responsive strains for CYP1A induction, and later the demonstration of reversible, saturable, and high affinity binding of radio-labeled TCDD to a soluble cytoplasmic protein of hepatocytes, provided pharmacological evidence that the *Ah* locus product functions as an “induction receptor”, designated as the aryl hydrocarbon receptor (AhR) (Poland and Glover, 1974; Poland and Glover, 1975; Poland et al., 1976). In this regard, AhR binds a CYP1A inducer and mediates the induction of the genes.

In addition to CYP1A induction, “other biological effects” of CYP1A inducers, typified by TCDD, also segregate with the high affinity AhR genotype, implicating AhR in a broader range of biological effects of the inducers than CYP1A induction. These effects include a wasting syndrome, thymic involution, altered proliferation and differentiation of epithelial cells, endocrine disorders, and tumor promotion, which were collectively known as the “adverse response” of halogenated aromatic hydrocarbons (HAH) to reflect the fact that they were observed in animals treated with a single dose of TCDD and related halogenated dibenzo-p-dioxins, dibenzofurans, and biphenyls (Poland and Knutson, 1982). It is plausible to postulate that the “adverse response” requires high affinity and persistent binding leading to prolonged activation of AhR by TCDD. On the other hand, PAHs are rapidly metabolized by elevated CYP1A1 activity, and therefore a single dose of PAH is unable to sustain the activation of AhR to launch the “adverse response”. In addition, TCDD and other HAHs are known to be metabolically resistant without forming reactive intermediates. Therefore, most of the effects of TCDD were thought to be mediated through AhR via an epigenetic mechanism(s).

DMD 15826

The availability of mouse strains with high and low affinity AhRs provided a unique approach to the analysis of CYP1A induction and PAH carcinogenicity and toxicity (Nebert, 1989) (Table 1). In addition to differences in the sensitivity of the strains for both induction and toxicity by PAH, apparent disparities were observed in the patterns of PAH-induced tumors and toxicity between *Ah* responsive (*b/b* or *b/d*) and non-responsive (*d/d*) mice: *b/b* and *b/d* mice are more prone than *d/d* mice to PAH-induced cancers and toxicity in target tissues in which an administered PAH is in direct contact with, such as the liver, skin, and lungs; whereas *d/d* mice are at greater risks than *b/b* and *b/d* mice of developing tumor and toxicity in organs distant to the site of administration, such as the bone marrow. Strikingly, B[a]p given in diet at a dose of 120 mg/kg/day caused all *d/d* mice to die within 3 to 4 weeks, while *b/b* or *b/d* mice survived the treatment for 6 months (Robinson et al., 1975). At low doses (6 or 12 mg/kg/day) of B(a)p, the *d/d* mice survived, but developed more leukemia than *b/b* or control mice (Nebert and Jensen, 1979). It was hypothesized that CYP1A induction (and consequently the metabolic activation and clearance of PAH) is high in the liver (and other sites of entry) in B6 mice resulting in a significant first pass effect of B[a]p metabolism, leading to tumor and toxicity at proximate sites. On the contrary, induction is weak in D2 mice, thus allowing more PAH to reach remote organs giving rise to increased tumor formation and toxicity at distant sites. This observation underscores the importance of both the dosage and route of administration in the evaluation of the impact of CYP1A induction on chemical toxicity. The findings suggest a protective role of CYP1A induction in the gut and/or liver against the bone marrow toxicity of high level oral B[a]p. However, the studies do not distinguish whether the phenotypic difference between the responsive and

DMD 15826

non-responsive mice is due to the low activities of CYP1A, the *Ah* locus product, or both in D2 mice. Thus, alternatively, activation of AhR or induction of target genes other than CYP1A may contribute to the observed differences in B[a]p toxicity between the *Ah^b* and *Ah^d* mice.

IV. Effect of CYP1A induction on toxicity and cancer: early *in vitro* and human studies

Since the early 1970s, a number of innovative *in vitro* approaches were developed and popularized rapid analyses of the carcinogenic and toxic effects of large numbers of chemicals under simple *in vitro* conditions. The application of these *in vitro* assays has led to several critical observations in the understanding of the interrelation between the biological effect of PAH and its metabolism by CYP1A (Table 1).

The “Salmonella/liver enzymes” (or “Ames”) test was employed to analyze the metabolic activation of 3-MC to mutagens with hepatic S-9 fractions from *b/b*, *b/d*, or *d/d* mice (Felton and Nebert, 1975). The findings revealed a correlation between induction of CYP1A1 enzyme activity and increased mutagenic activity of 3-MC. Many other PAHs were found to require bioactivation by CYP1A for their mutagenic effects in the “Ames” test as well, consistent with a linear mechanistic linkage among CYP1A induction, metabolic activation, mutagenesis, and tumorigenicity of PAHs.

The V79 Chinese hamster cell line contains the NADPH cytochrome P450 reductase but lacks P450-dependent activities. Genetic engineering of the cells with CYPs reconstitutes the metabolic capability thus permitting genotoxicity testing, in which mutagenic metabolites are both generated and detected in one cell type (Ellard et al., 1991). V79 cells have also been widely used in co-cultivation experiments with liver

DMD 15826

enzymes. By comparing the genotoxicity of PAH in V79 cells expressing different CYPs, 3-MC was found to depend on CYP1A1 for its mutagenicity, while 2-aminoanthracene requires CYP1A2 and an acetyltransferase activity to elicit high mutagenic response. Moreover, tobacco particulates were found to induce a greater micronuclei formation in cells expressing CYP1A1 compared with V79 cells lacking the enzyme, implicating PAH, in addition to direct-acting components, in the genotoxicity of tobacco particulate matter (Ellard et al., 1991).

The 1970s were also marked with the elucidation of the chemical structures of the metabolites and the pathways of bioactivation of PAHs. More importantly, through the use of a combination of *in vitro* (“Ames” test, V79 culture, and rat microsome or P450 fractions) and *in vivo* assays, the trans-7,8-diol 9,10-epoxide metabolite of B[a]p was found to be the major metabolic product of the rat CYP1A1 enzyme and the principal ultimate mutagen and carcinogen of B[a]p (Conney, 1982; Jerina, 1983; Miller and Ramos, 2001). From these studies, the concepts of pro, proximate, and ultimate carcinogens were developed. The studies also established the induction of the CYP1A1 enzyme activity as a primary event in the formation of the trans-7,8-diol 9,10-epoxide of B[a]p.

The consequence(s) of B[a]p bioactivation by CYP1A in cultured cells was best illustrated in the selection of B[a]p resistant variants in hepa1c1c7 cells, a mouse hepatoma cell line that preserves many of the hepatocyte properties and is highly responsive to induction of CYP1A by PAH inducers (Whitlock, 1990; Hankinson, 1995). Upon exposure to B[a]p, most hepa1c1c7 cells died, presumably due to the toxicity of trans-7,8-diol 9,10-epoxide formed from B[a]p, correspondent to increased DNA and

DMD 15826

protein adduct formation. A few resistant variants, however, grew in the presence of B[a]p. Complementation experiments separated the variants into at least three complementation groups that were later defined as the genes encoding CYP1A1, AhR, and Arnt, respectively. Thus, a transcription circuit is created: B[a]p activates AhR and Arnt to mediate the induction of CYP1A1, which metabolize B[a]p to toxic intermediates, leading to cell death. The findings established that induction of CYP1A1 is a primary determinant of B[a]p toxicity in cultured cells.

Analyses of the induction of AHH activity in human tissues or cultured human cells, provided the initial observations of CYP1A induction in humans. Early studies by Conney and associates first revealed that AHH activity was detected in placentas obtained after birth from smoking mothers, but not from non-smokers, consistent with an induction of CYP1A by PAHs present in tobacco smoke (Welch et al., 1969). However, AHH activities in placentas from mothers who smoke the same amount of cigarettes exhibit variations as large as 84-fold. In cultured neonatal human foreskin cells, basal AHH activities were detected with a 3-fold variability. The activity was inducible by benz[a]anthracene with a large variation (from 180 to 530%) among the testing individuals (Alvares et al., 1973).

By using a mitogen-activated, PAH-treated human lymphocyte culture system, Kellermann et al (Kellermann et al., 1973a) observed that the normal white population in the United States can be divided into three distinct groups with low, intermediate, and high degrees of inducibility of AHH activity. Phenotype frequencies were 53 percent, 37 percent, and 10 percent, respectively. Furthermore, by using the same assay, the same

DMD 15826

group found a close association between the susceptibility to bronchogenic carcinoma and high inducibility of AHH activity in 50 cancer patients (Kellermann et al., 1973b).

These studies not only revealed large variations in the induction of CYP1A in human populations, but also suggest a causative role of CYP1A induction in the occurrence of cancer in certain human populations. Subsequent reports provided evidence supporting a correlation between high CYP1A inducibility and cancer of the lung, larynx, and oral cavity that are in direct contact with cigarette smoke. However, it is noteworthy to point out that no association between lung cancer and CYP1A inducibility has been reported in a number of studies (Ma and Lu, 2003).

Together, from these early *in vitro*, mechanistic, and human studies, it appears to be logical to conclude that induction of CYP1A would impose detrimental effects upon individuals by way of metabolic activation of PAH and other chemicals to ultimate carcinogens or reactive toxic intermediates.

V. The Ah Receptor

A major obstacle to the study of the Ah receptor in the 1970s and 1980s was the difficulty in purifying the protein to homogeneity. By using 2-azido-3-[¹²⁵I]iodo-7,8-dibromodibenzo-*p*-dioxin, a photoaffinity ligand of AhR, Bradfield et al (Bradfield et al., 1991) were able to covalently label the Ah receptor from the liver of C57BL/6J mice and purify the protein to apparent homogeneity under denaturing conditions. The purification procedure involved two ion exchange chromatography steps, which gave ~100 fold enrichment and 40-50% recovery, followed by three rounds of C4 reverse phase HPLC, to eventually reach a >150,000-fold purification with an overall yield of 3-5%. The putative amino-terminal sequence of the AhR peptide was determined from this

DMD 15826

preparation by peptide sequencing. A synthetic peptide based on the N-terminal amino acid sequence was used to raise polyclonal antibodies. The antibodies recognized both denatured and non-denatured photoaffinity-labeled Ah receptor from C57BL/6J mouse liver as a 95 KD protein (Poland et al., 1991). The antibodies also recognized AhR from chicken, rodents, monkey, and human, indicating conservation of the N-terminal epitope.

Cloning of the AhR cDNA (*b-1* allele) using degenerate oligo probes derived from the putative N-terminal peptide sequence revealed that AhR contains 805 amino acid residues with a calculated molecular weight of 89,426 Dalton (Burbach et al., 1992). Importantly, the N-terminal region of AhR contains a stretch of basic residues followed by a helix-loop-helix turn motif; the structure is termed “bHLH” and is commonly seen among members of the bHLH family of transcription factors. The bHLH motif functions as the DNA-binding and protein dimerization domain of the transcription factors. Additionally, the AhR protein contains two imperfect inverted 51-amino acid repeats adjacent to bHLH that were also found in Per, which is a circadian transcription factor, Arnt (aryl hydrocarbon receptor nuclear translocator), which is the dimeric partner of AhR for CYP1A transcription, and Sim, which is the *Drosophila* “single-minded” protein. The motif was named as the “PAS” motif (Hoffman et al., 1991). The “PAS” motif of AhR was found to involve ligand binding, binding with the hsp90 protein in the cytoplasm, and dimerization of AhR with Arnt in the nucleus. The carboxyl half of AhR contains three separable transcription activation domains that are acidic, serine/threonine-rich, and glutamine-rich, respectively (Ma et al., 1995). Thus, AhR was formally classified as a ligand-activated transcription factor of the bHLH-PAS family.

DMD 15826

The human AhR is about 10-fold less sensitive to the induction of AHH activity by TCDD than that of B6 AhR in parallel with a 10-fold lower binding affinity for TCDD, resembling the *Ah* non-responsive phenotype (Harper et al., 1988). Cloning of the human AhR cDNA revealed that it encodes a protein of 848 amino acid residues (Ema et al., 1994). Structurally, the human AhR is more similar to the D2 AhR than the B6 AhR with two critical determinants reducing ligand binding affinity observed in D2 AhR: a T to G mutation at the position equivalent to the termination codon (TGA) of the B6 AhR causing an elongation of the carboxyl terminus and a Val³⁸¹ equivalent to the Val³⁷⁵ of D2 AhR replacing Ala³⁷⁵ of B6 AhR. Scatchard plot analysis of expressed human AhR gave a K_d value of 1.58 nM for TCDD in agreement with that of D2 AhR (1.66 nM), ~ 6 fold higher than that of B6 AhR (0.27 nM); the K_d values of the mouse AhRs are qualitatively similar to those reported earlier (16 nM for D2 and 1.8 nM for B6) (Okey et al., 1989).

VI. The CYP1A knockout mouse models

The construction of targeted knockout mouse models of the *cyp1a1*, *cyp1a2*, *cyp1b1*, and double knockout of the genes made it possible to directly evaluate the role of CYP1A in chemical toxicity and carcinogenesis in intact animals. Distinctly contradictory to the observations from *in vitro* studies that CYP1A enhances the toxicity of PAHs, the intact animal experiments after oral dosing revealed an essential protective role of CYP1A induction in PAH toxicity (Nebert et al., 2004) (Table 1). An oral dose of B[a]p at 125 mg/kg/day caused death of all *cyp1a1*^{-/-} mice within 30 days; whereas no mortality or apparent signs of toxicity were observed in *cyp1a1*^{+/+} mice receiving the same treatment (Uno et al., 2004). Pathological lesions observed include striking

DMD 15826

decreases in the sizes of spleen and thymus, leukocytopenia, and extreme hypocellularity of the bone marrow in *cyp1a1*^{-/-} mice that may have caused the lethality. Increased toxicity of B[a]p in *cyp*^{-/-} mice correlated with a slower clearance rate (4-times slower), but a higher amount of B[a]p-DNA adduct formation, typically in the liver, spleen, and bone marrow, but not the small intestine, in *cyp1a1*^{-/-}, but not *cyp1a1*^{+/+} mice,.

Protection against B[a]p-induced mortality by CYP1A1 was not observed when B[a]p was administered at 125 mg/kg via i.p. injection.

A similar experiment was conducted comparing *cyp1a1*^{-/-}, *cyp1a2*^{-/-}, *cyp1b1*^{-/-}, *cyp1a1*^{-/-}/*cyp1b1*^{-/-}, *cyp1a2*^{-/-}/*cyp1b1*^{-/-}, and *cyp1*^{+/+} mice (oral B[a]p of 125 mg/kg/day for 18 days) (Uno et al., 2006). Marked wasting, immunosuppression, and bone marrow hypocellularity were observed only in *cyp1a1*^{-/-} mice. Together, these studies provide a conclusive proof of a protective role of CYP1A1 induction in the intestine and/or liver against the immune and bone marrow toxicity of oral B[a]p. In addition, the studies revealed that the immune and bone marrow toxicity, but not the thymic atrophy and hepatocyte hypertrophy effects, of oral B[a]p required CYP1B1 and the magnitude of the immune damage can be independent of total plasma B[a]p concentration and clearance.

The *cyp1a2*^{-/-} mice exhibited increased toxicity from drugs that are predominantly CYP1A2 substrates. Paradoxical results were observed concerning the role of CYP1A2 in the tumorigenicity of 4-aminobiphenyl (ABP) (Tsuneoka et al., 2003). CYP1A2 enhanced the metabolic activation, ABP-DNA adduct formation, and toxicity *in vitro*. However, *cyp1a2*^{-/-} mice exhibited increased adducts in the liver and urinary bladder by topical ABP; ABP-induced hepatocellular carcinomas and preneoplastic foci as well as ABP-induced methemoglobinemia were increased in the

DMD 15826

mice. Similar paradoxical roles of CYP1A2 were observed in the DNA adduct formation and malignancies induced by 2-amino-3-methylimidazo[4,5*f*]quinoline (IQ) and 2-amino-1-methyl-6-phenyl-imidazo[4,5*b*]pyridine (PhIP) (Snyderwine et al., 2002; Kimura et al., 2003). The results demonstrate alternative pathways of metabolic activation of ABP, IQ, and PhIP in the absence of CYP1A2 and protection against adduct formation of the chemicals by CYP1A2 in intact animals.

The more “stable” ligands of AhR, exemplified by TCDD, can cause a broad range of “adverse response” in animals in an AhR-dependent manner in addition to CYP1A induction. The mechanism of the toxic effects of TCDD is not well understood. TCDD is a poor substrate of CYP1A and does not form reactive intermediates. The interrelation between CYP1A induction and TCDD toxicity remains a subject of debate. The teratogenic effect of TCDD (cleft palate formation and hydronephrosis) was assessed in *cyp1a1*^{-/-}, *cyp1a2*^{-/-}, and *cyp1b1*^{-/-} mice (Dragin et al., 2006). TCDD at 25 µg/kg by gavage on gestation day 10 was found to be lethal to fetuses carried by *cyp1a2*^{-/-}, but not *cyp1a1*^{-/-} or *cyp1b1*^{-/-}, dams. Fetuses from *cyp1a2*^{-/-} dams exhibited a 6-fold increase in sensitivity to cleft palate, hydronephrosis, and lethality. The effect was dependent on the maternal *cyp1a2*^{-/-} genotype and correlated with more TCDD reaching the embryos from *cyp1a2*^{-/-} dams. High levels of TCDD were found in adipose tissue, mammary gland, and blood of the *cyp1a2*^{-/-} mothers, compared with the high level of TCDD in the livers of the *cyp1a2*^{+/+} mothers. The increased sensitivity to TCDD-induced birth defect was reverted by expressing human CYP1A1 and 1A2 in *cyp1a2*^{-/-} mice. Since TCDD binds to CYP1A2 to an appreciable amount in the liver, the results support the notion that maternal mouse hepatic CYP1A2 and its induction protects the embryos from TCDD-

DMD 15826

induced toxicity and teratogenic effects by way of sequestering TCDD in the maternal liver.

VII. The omeprazole story

Omeprazole (brand name: Prilosec, Rapinex) is a proton pump (H^+ , K^+ -ATPase) inhibitor used for its high efficiency as a blocker of gastric acid secretion in gastric parietal cells. Omeprazole is used clinically for the treatment of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease (GORD/GERD), and the Zollinger-Ellison syndrome. A major pathway of metabolism of omeprazole is its conversion to 5-hydroxy-omeprazole by CYP2C19 (Andersson, 1996). Metabolism to 5-O-desmethyl-omeprazole by CYP2C19, and to 3-hydroxy-omeprazole and omeprazole sulfone by CYP3A constitutes the minor metabolic pathways of the drug.

Although omeprazole is not a substrate of CYP1A, it induced CYP1A1/2 in primary human hepatocytes at mRNA, protein, and enzyme (phenacetin deethylase, acetanilide hydroxylase, benzpyrene hydroxylase, and ethoxyresorufin deethylase) levels (Diaz et al., 1990). Induction of the CYP1A2 protein was from 4 to 50 fold, comparable to induction by 3-MC or β -naphthoflavine (β NF) in cultured human hepatocytes. Moreover, 2 to 10 fold induction of the CYP1A2 protein and CYP1A-dependent activities was observed *in vivo* in liver biopsies from cancer patients before and after 4 day-treatment with omeprazole at therapeutic doses. The study established omeprazole as the first clinical drug to be characterized as an inducer of CYP1A in the human liver (Diaz et al., 1990).

The 5-hydroxylation of omeprazole by human liver microsomal preparations and the *in vivo* clearance of omeprazole in humans exhibit a large inter-individual variation

DMD 15826

that correlates with the polymorphic phenotypes of *S*-mephenytoin hydroxylase (catalyzed by CYP2C19) (Sohn et al., 1992; Chiba et al., 1993). Differences in the rate of 5-hydroxylation between poor metabolizers (PM) and extensive metabolizers (EM) results in different tissue levels of omeprazole available for CYP1A induction (Tang et al., 2005). At a therapeutic dose (40 mg), omeprazole failed to induce CYP1A2 as measured by a caffeine *N*-3-demethylation breath test in individuals with EM phenotype, but the induction was revealed at a higher dose (120 mg) in the same individuals. On the other hand, induction of CYP1A2 by omeprazole was observed in individuals with PM phenotype at the dose of 40 mg. Clearly, individual variations in the metabolic rate of omeprazole affect the intracellular concentration of the inducer contributing to variability of CYP1A induction (Rost et al., 1992; Rost et al., 1994).

In several clinical studies, induction of CYP1A2 by omeprazole was examined using CYP1A2 marker drugs (caffeine and phenacetin) in human volunteers who were not phenotyped for CYP2C19. Induction of caffeine and phenacetin metabolism by CYP1A2 was not observed (Andersson et al., 1991; Xiaodong et al., 1994; Rizzo et al., 1996). It is noteworthy that these studies were conducted in White populations, in which only a small portion of the population are PM, while most individuals are EM. Therefore, induction of CYP1A can not be observed with 40 mg of omeprazole in these populations. Cancer patients in general have a decreased capacity of drug metabolism; therefore, many of them represent PM subjects. This notion may explain why marked induction of CYP1A by omeprazole was observed in the study of cancer patients by Diaz and associates discussed above (Diaz et al., 1990).

DMD 15826

The finding of CYP1A induction by omeprazole in humans raised an issue of considerable clinical importance: induction of CYP1A by clinical drugs that are not known to be metabolized by CYP1A many induce unexpected drug-drug interactions (DDI); moreover, induction may exhibit high individual variability making it more difficult to predict the impact of the induction on drug-drug interactions.

Since CYP1A2 is the major phenacetin deethylase in humans, induction of the enzyme by omeprazole would increase the metabolism of phenacetin to acetaminophen and further to the formation of *N*-acetyl-*p*-benzoquinoneimine that is responsible for liver necrosis induced by the drugs. Based on this knowledge, it can be argued (Farrell and Murray, 1990) that acetaminophen liver toxicity could be increased in patients receiving both omeprazole and phenacetin or acetaminophen (two widely used analgesic drugs). Induction of CYP1A1 by omeprazole in patients who smoke may increase the chance of lung cancer due to increased metabolic activation of PAHs in the tobacco smoke by CYP1A1. In both scenarios, individuals with a PM phenotype of CYP2C19 are expected to have higher tissue concentrations of omeprazole, increased induction of CYP1A, and more formation of toxic and/or carcinogenic intermediates of phenacetin, acetaminophen, or PAHs from CYP1A leading to increased toxicity or tumorigenicity than individuals with an EM CYP2C19 phenotype. On the other hand, CYP1A2 catalyzes the 4-hydroxylation of aflatoxin B₁, which represents an efficient pathway of detoxification of B₁. Induction of CYP1A2 would increase metabolic detoxification of aflatoxin B₁ resulting in decreased carcinogenicity of the compound (Koser et al., 1988). Thus, long term treatment with omeprazole may provide a protective effect against aflatoxin B₁-induced carcinogenesis in certain countries and regions where B₁-induced liver cancer is

DMD 15826

a particular public health concern. However, clinical data supporting this notion is not available at the present.

VIII. CYP1A induction and drug development

The realization of the prominent role of inducible CYP1A1 in the metabolic activation of PAHs and that of CYP1A2 in the activation of HAA, phenacetin and other drugs led to a widely held belief that induction of CYP1A is detrimental to humans and animals in general. This notion had a broad impact on chemical safety evaluation, in particular, in the area of carcinogenesis and environmental health. The role of CYP1A induction in drug development remains debatable and warrants certain clarification.

In two recent publications concerning CYP1A induction, it was stated that pharmaceutical companies employ a general policy for several decades that, in routine drug development testing, if a candidate drug shows CYP1 inducibility, further testing is generally discontinued for fear of possible toxic or carcinogenic effects (Nebert et al., 2004; Uno et al., 2004). However, the two references cited to support this argument apparently did not address CYP1 induction as a general policy in drug screening (Valles et al., 1995; Gastel, 2001). There is a lack of supporting evidence for the statement in literature as well.

It is our understanding that, although potential induction of drug-drug interaction and bioactivation of toxic or carcinogenic compounds due to the induction of CYP1A1/1A2 have been a concern of safety in drug development, induction of CYP1A was rarely the deciding factor to determine whether a compound should be dropped for further testing because of its inducibility of CYP1A. In fact, omeprazole has been shown to be a CYP1A2 inducer both in cultured human hepatocytes and in the human liver

DMD 15826

(Diaz et al., 1990). Yet it was approved by Regulatory Agencies worldwide and has been used safely for more than 20 years by millions of people for clinical treatment of gastric ulcer and related disorders.

In the practice of drug development, it is recognized that induction of CYP1A2 can affect the drug level of co-administered drugs that are metabolized by CYP1A2. The issue is treated like the induction of other P450 enzymes such as CYP3A4. Drug-drug interactions can be evaluated by *in vitro* metabolism studies, and by clinical DDI studies. The drug candidate is only dropped if it has very serious safety issues and clinically relevant DDI effects.

The clinical use of omeprazole provided an example of cancer risk evaluation of CYP1A inducers in drug development (Farrell and Murray, 1990). The CYP1A inducibility of omeprazole in humans and its inter-individual variability associated with CYP2C19 phenotypes were well documented. Increased risk of malignancy in patients taking omeprazole is theoretically possible due to the critical role of CYP1A in the metabolic activation of PAH and other carcinogens. On the basis of these considerations, it was suggested that patients for whom long-term administration of omeprazole is highly desirable should minimize exposure to cigarette smoke and dietary sources of PAHs such as charboiled food and continued long-term surveillance of such individuals for potential increase of malignancy was recommended (Farrell and Murray, 1990). However, the results from knockout mice and the safety record of omeprazole used by millions of individuals in over twenty years have not yet shown an etiological connection between omeprazole use and cancer incidence.

IX. Conclusion

DMD 15826

The discovery of induction of AHH (CYP1A) in the 1950s led to the uncovering of a principal mechanism of regulation of drug metabolism. Moreover, studies of CYP1A induction had profound influence in broad areas of biomedical sciences including drug metabolism and drug development, cancer research, pharmacology, toxicology, and safety regulation of therapeutic agents, and environmental and occupational chemicals. With regard to human safety evaluation, the research of CYP1A induction not only played a critical role in the understanding of the carcinogenicity and toxicity of PAH and other CYP1A substrates, but also served as a model system for analyzing the interrelations between induction of drug metabolism and chemical toxicity in general. Interestingly, from the early discovery of protection against chemical carcinogenesis via the induction of AHH by PAHs in *in vivo* studies in the 50s and 60s, to the elucidation of the mechanism and biological implication of the metabolic activation of PAH procarcinogens to ultimate carcinogen (reactive intermediate metabolite) by CYP1A1/2 using *in vitro* approaches in the 70s and 80s, and to the demonstration of the protective role of CYP1A in B[a]p toxicity with the use of targeted gene knockout mice of the CYP1A genes in recent years, our understanding of CYP1A induction in chemical safety appears to have undertaken a full circle.

Two important lessons can be learned from the CYP1A induction experience for safety evaluation. Firstly, *in vitro* studies permit mechanism-based analysis of a biological process at molecular levels, which can often be performed quickly and in large scale allowing rapid analysis of large numbers of chemicals. Whereas, *in vivo* studies reflect intact animal response, but are often time-consuming, expensive, limited in the number of chemicals to be analyzed, and difficult in detailed mechanistic analysis. The

DMD 15826

uncovering of the critical role of the first pass metabolism of PAH in intestine/liver by CYP1A in PAH toxicity and carcinogenesis clearly demonstrates the notion that precautions must be taken in the safety evaluation of CYP induction when extrapolating *in vitro* results to intact animals, as metabolism by CYP is often dependent upon organ, tissue, route and time of administration, age, gender, co-administered drugs or agents, and certain pathophysiological conditions. Secondly, from the early demonstration of large inter-individual variation in AHH induction in placenta of smoking mothers to the recent observation of the association of CYP1A induction by omeprazole with the PM CYP2C19 phenotype, human studies of CYP1A induction have uncovered multiple genetic and environmental factors influencing CYP1A inducibility and its impact in PAH carcinogenicity in humans. Thus, extrapolation of results from animal studies of CYP induction to humans is a complex process that requires, ultimately, the direct proof from human studies. Epidemiological studies of exposed populations may help address the human relevance of animal studies.

DMD 15826

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

DMD 15826

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Table 1 Comparison of major B[a]p responses *in vitro* and *in vivo*^a

	Molecular Events	End Responses
<i>In vitro</i>	Binding to AhR Induction of CYP1A1 Metabolic activation of B[a]p ↑ trans-7,8-diol 9,10 epoxide ↑ DNA & protein adduct formation	↑ Mutation in “Ames” test ↑ Micronuclei formation in V79 cells ↑ Apoptosis and cell death of cultured cells Identification of genetic B[a]p-resistant variants
<i>In vivo</i> ^b		
<i>Ah</i> responsive (<i>b/b</i> or <i>b/d</i>)	Strong induction of CYP1A1 & metabolism at site of entry ↑ First pass detoxification	Tumors and toxicity at proximate tissues Survival for > 6 month
<i>Ah</i> non-responsive (<i>d/d</i>)	Weak metabolism at site of entry ↓ First pass detoxification	Profound toxicity at distal tissues: bone marrow depression, leukocytopenia, & wasting Death within 30 days ↑ Incidences of leukemia and thymomas at lower dose of B[a]p (12.5 mg/kg)
<i>cyp1a1</i> ^{-/-}	Similar to <i>d/d</i>	Profound toxicity and death similar to <i>d/d</i>

^a Summarized from a number of references (Robinson et al., 1975; Nebert and Jensen, 1979; Conney, 1982; Nebert, 1989; Whitlock, 1999; Uno et al., 2004; Uno et al., 2006).

^b Represent experiments in which B[a]p was given in corn oil-soaked food at daily oral B[a]p dose of ~125 mg/kg/day for most responses or ~12.5 mg/kg for tumor formation.