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Oral Exposure to Benzo[*a*]pyrene in the Mouse: Detoxication by Inducible Cytochrome P450 Is More Important Than Metabolic Activation

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ABSTRACT

The cytochrome P450 (CYP1A1) enzyme metabolically activates many polycyclic aromatic hydrocarbons, including benzo[*a*]pyrene (BaP), to DNA- and protein-binding intermediates that are associated with toxicity, mutagenesis, and carcinogenesis. As a result, it is widely accepted that CYP1A1 potentiates the toxicity of this class of chemicals. In distinct contrast, we show here that CYP1A1 inducibility is essential in the detoxication of oral BaP. We compared *Cyp1a1*(−/−) knockout mice, having the genetic absence of the CYP1A1 enzyme, with *Cyp1a1*(+/+) wild-type mice. At an oral BaP dose of 125 mg/kg/day, *Cyp1a1*(−/−) mice died within 30 days whereas *Cyp1a1*(+/+) mice displayed no outward signs of toxicity. The rate of BaP clearance was 4-fold slower in *Cyp1a1*(−/−) than *Cyp1a1*(+/+) mice. The cause of death in *Cyp1a1*(−/−) mice receiving oral BaP seemed to be immunotoxicity, including toxic chemical depression of the bone marrow; some toxic effects in *Cyp1a1*(−/−) mice were noted at a BaP dose as low as 1.25 mg/kg/day. DNA post-labeling studies demonstrated dramatically higher BaP-DNA adduct levels in all *Cyp1a1*(−/−) tissues assayed, with the exception of the small intestine, which is probably a major site of BaP metabolism in *Cyp1a1*(+/+) mice. Different BaP-DNA adduct patterns were also observed between the two genotypes receiving oral BaP. Despite previous studies in vitro and in cell culture that have shown a participatory role for CYP1A1 in BaP toxicity, the present data indicate that, in the intact animal, inducible CYP1A1 is extremely important in detoxication and protection against oral BaP toxicity.

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental contaminants especially found in the byproducts of combustion processes such as coke ovens, cigarette smoke, and charcoal grilling of food. An important and very extensively studied prototype of this class of compounds is benzo[*a*]pyrene (BaP) (Pelkonen and Nebert, 1982; Conney et al., 1994; Knize et al., 1999; Miller and Ramos, 2001). BaP has been demonstrated to cause carcinogenic, mutagenic, cytotoxic, and teratogenic effects in various species and tissues (Nebert, 1989; Ellard et al., 1991; Conney et al., 1994; Miller and Ramos, 2001). BaP is implicated as a causative agent in lung carcinogenesis (Alexandrov et al., 2002) and atherosclerosis (Miller and Ramos, 2001) as a result of cigarette smoking.

PAHs such as BaP must be metabolically activated to exert their toxic effects. The etiology of toxicity and cancer caused by BaP and other PAHs is complex because: 1) PAHs are metabolically activated by phase I enzymes to reactive intermediates that bind covalently to nucleic acids and proteins, 2) PAHs are detoxified by both phase I and phase II enzymes, 3) PAHs induce numerous enzymes involved in activation and detoxication of PAHs via the aromatic hydrocarbon receptor (AHR); and 4) PAHs affect the expression of multiple other genes by way of both AHR-dependent and -independent mechanisms (Nebert, 1989; Puga et al., 1992; Ryu et al., 1996; Delescluse et al., 2000; Nebert et al., 2000b; Puga et al., 2000; Miller and Ramos, 2001; Mimura and Fujii-Kuriyama, 2003).

PAH-inducible cytochromes P450 include CYP1A1 (Nebert, 1989), CYP1A2 (Landi et al., 1999), CYP1B1 (Spink

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ABBREVIATIONS: PAH, polycyclic aromatic hydrocarbon; BaP, benzo[*a*]pyrene; AHR, aromatic hydrocarbon receptor; PTGS2, prostaglandin G synthase-2 (cyclooxygenase-2); TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; AUC, area under the curve; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

et al., 2002), cytochrome P450 2A (Kimura et al., 1989), cytochrome P450 2C (Fisslthaler et al., 1999), and cytochrome P450 2S1 (Rivera et al., 2002). PAHs such as BaP are known to be potent substrates for CYP1A1, CYP1B1, and cyclooxygenase-2 (PTGS2) (Marnett et al., 1982), whereas arylamines are the usual substrates for CYP1A2. CYP1A2 is constitutively high in mammalian liver. Although CYP1B1 has a high basal activity in such tissues as adrenal, ovary, testis, prostate, and mammary gland, gastrointestinal tract, and the immune system (Walker et al., 1995), constitutively CYP1B1 is extremely low but inducible in liver and inducible in the gastrointestinal tract after PAH treatment (Buesen et al., 2002; Zhang et al., 2003). CYP1A1, on the other hand, is constitutively nil but ubiquitous after induction by PAHs (Dey et al., 1999). Hundreds of studies performed in vitro and in cell culture have demonstrated clearly that CYP1A1 is involved in the metabolic activation of BaP into reactive intermediates capable of binding to DNA and proteins. Numerous reports have shown that one or more of the reactive intermediates, rather than the nonmetabolized parent compound, is responsible for BaP-mediated toxicity, mutations, cancer, and birth defects (Pelkonen and Nebert, 1982; Conney et al., 1994; Knize et al., 1999; Miller and Ramos, 2001).

To examine the role of CYP1A1 in the intact animal, we compared the *Cyp1a1*(−/−) knockout mouse (Dalton et al., 2000) with *Cyp1a1*(+/+) wild-type mice. In hepatotoxicity experiments after very large doses of i.p. BaP (Uno et al., 2001), we had expected that the *Cyp1a1*(−/−) mouse would be more protected than the *Cyp1a1*(+/+) wild-type mouse against liver damage and hepatic BaP-DNA adduct formation; to our surprise, just the opposite was found. In the present study, we have extended this work by comparing oral (p.o.) versus intraperitoneal (i.p.) administration of BaP at three doses.

Materials and Methods

Chemicals. BaP was purchased from Sigma-Aldrich (St. Louis, MO). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was purchased from Accustandard, Inc. (New Haven, CT). All other chemicals and reagents were obtained from either Aldrich Chemical Co. (Milwaukee, WI) or Sigma-Aldrich as the highest available grades. Anti-CYP1A1/1A2 and anti-CYP1B1 polyclonal antibodies were purchased from BD Gentest (Woburn, MA). The anti-PTGS2 polyclonal antibody was a generous gift from Larry Marnett (Vanderbilt University, Nashville, TN).

Animals. Generation of the *Cyp1a1*(−/−) mouse line, starting from the C57BL/6J and 129/J inbred strains, has been described previously (Dalton et al., 2000). The *Cyp1a1*(−/−) colony that we maintain has been backcrossed into C57BL/6J for eight generations; this ensures that the knockout genotype resides in a genetic background that is >99.8% C57BL/6J (Nebert et al., 2000a). Age-matched C57BL/6J *Cyp1a1*(+/+) wild-type mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All animal experiments were approved by, and conducted in accordance with, the National Institutes of Health standards for the care and use of experimental animals and the University of Cincinnati Medical Center Institutional Animal Care and Use Committee.

Biohazard Precaution. BaP and TCDD are very toxic and probably human carcinogens. All personnel were instructed in safe handling procedures. Lab coats, gloves, and masks were worn at all times, and contaminated materials were collected separately for disposal by the Hazardous Waste Unit or by independent contractors. BaP- and TCDD-pretreated mice were housed separately, and their carcasses were treated as contaminated biological materials.

Treatment. Preliminary experiments showed no substantial differences between male and female mice, so one gender (male) was chosen for all subsequent studies. BaP treatment experiments were always begun with *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice at approximately 4 weeks of age. In the survival experiments, we gave i.p. BaP (125 mg/kg/day, in corn oil, 25 ml/kg). For the pharmacokinetics studies, BaP (15 mg/kg) was given by gavage, and BaP (125, 12.5, or 1.25 mg/kg) was given p.o. In all other experiments, BaP was given in corn oil-soaked food. The rodent food (Harlan Teklad, Madison, WI) was soaked at least 24 h in BaP-containing corn oil (10, 1.0, or 0.1 mg/ml). By knowing the weight of the food ingested daily by a 20-g mouse and by using [^{3}H]benzo[a]pyrene in several experiments (Robinson et al., 1975), the daily oral BaP doses were approximately 125, 12.5, and 1.25 mg/kg/day. To start day 1 of the experiment, the mice were presented with the BaP-laced food; control mice received food soaked in corn oil alone. The mice eagerly ate the corn oil-soaked food. Oral BaP experiments usually were run for 5 or 18 days. All tissues were harvested between 9:00 and 10:00 AM to exclude any circadian rhythm effects.

Pharmacokinetic Studies. A BaP dose of 15 mg/kg body weight (in 150 μl) was given by gavage to *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice, and sequential blood samples were drawn from the saphenous vein of the same mouse by the procedure described previously (Hem et al., 1998). Because of marked differences in clearance time noted between the genotypes in preliminary experiments, blood was drawn from the same *Cyp1a1*(−/−) mouse at 0.5, 1.0, 1.5, 2, 3, 4, 6, 9, 12, and 15 h; blood was drawn from the same *Cyp1a1*(+/+) mouse at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, and 6 h. Each time with each mouse, at least 20 μl of blood was collected to analyze 15 μl of whole blood. Some mice were treated i.p. with TCDD (15 μg/kg body weight) in corn oil vehicle 48 h before BaP administration by gavage. The following principal pharmacokinetic parameters were determined using the MW£harm software (MW£harm, version 3.50; MediWare, Groningen, The Netherlands): elimination half-life, total body clearance, and area-under-the-curve (AUC). This software package was provided by Sander Vinks (Cincinnati Childrens Hospital, Cincinnati, OH). In addition to BaP by gavage, we attempted intravenous BaP pharmacokinetics but found this to be technically too difficult; veins of the *Cyp1a1*(−/−) mouse seemed to collapse far more easily than veins of the *Cyp1a1*(+/+) wild-type animal.

Detection of BaP in Blood. We measured BaP levels in blood by modifying methods described previously (Garcia-Falcon et al., 1996; Kim et al., 2000). Whole blood (15 μl) was extracted three times with an ethyl acetate/acetone mixture (2:1, v/v). The organic extracts were pooled and dried under argon, and the residue was resuspended in 250 μl of acetonitrile. An aliquot (100 μl) aliquot was injected onto a Nova-Pak C_{18} reverse-phase column (4 μm, 150 × 3.9-mm i.d.; Waters, Milford, MA). High-performance liquid chromatography analysis was conducted on a Waters model 600 solvent controller, equipped with a fluorescence detector (F-2000; Hitachi Software Engineering, Yokohama, Japan). Isocratic separation was performed using an acetonitrile/water (85:15, v/v) mobile phase at a flow rate of 1 ml/min. The excitation and emission wavelengths were 294 and 404 nm, respectively. The BaP concentrations in blood were calculated by comparing the peak amplitude of samples with those of control blood spiked with different known concentrations of BaP; the calibration curve for BaP showed excellent linearity (correlation coefficient $r > 0.998$). Four BaP metabolites were found to run far ahead of BaP on the column and thus did not interfere. The detection limit (defined as 3 times the signal-to-noise ratio) was 0.05 mg/ml, and the limit of BaP quantification was determined to be 0.2 ng/ml. The intraday and interday precision of repeated analyses ($n = 4$) gave us coefficients of variation of <12%.

Plasma Enzymes. Levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, in plasma previously frozen at −20°C, were determined, using kits purchased from Sigma-Aldrich.

Histology. Blood samples were air dried on glass slides. Bone marrow was obtained at sacrifice by dissecting the femurs free of muscle, removal of the proximal and distal epiphyses, and a tiny polyethylene tube was affixed to one end; the marrow was gently blown onto a glass slide, and a second slide was used to squash the droplet of marrow onto the slide. These slides were then air dried. All slides were stained with Wright-Giemsa stain (University Hospital Bone Marrow Lab).

Differential counts of the peripheral blood and marrow were performed. The myeloid line included: promyelocytes, myelocytes, promegakaryocytes, megakaryocytes, neutrophils, eosinophils, basophils, and monocytes. The lymphoid line included: large, medium, and small lymphocytes and plasma cells. The erythroid line included: basophilic erythroblasts, polychromatophilic erythroblasts, orthochromatic erythroblasts, and red blood cells. Percentages of different cell types were calculated, based usually on 1000 cells tabulated, and the differentials were always counted on a minimum of 100 lymphocytes per animal.

Microsomal Protein Immunoblots. Microsomes (S9 fraction) from liver, small intestine (2 cm extending distally from the pylorus), spleen, and bone marrow were prepared as described previously (Uno et al., 2001). Protein concentrations were determined using the Bio-Rad protein reagent (Bio-Rad, Hercules, CA). Microsomal proteins were separated on SDS (0.1%)-polyacrylamide (10%) minigels. Separated proteins were transferred to nitrocellulose and visualized with Ponceau S to verify equivalent loading across lanes. Western blot analysis was performed using goat polyclonal anti-human CYP1A1/1A2 and polyclonal anti-human CYP1B1 (both from BD Gentest), and rabbit anti-mouse PTGS2 (generous gift of Larry Marnett, Vanderbilt University). Horseradish peroxidase-conjugated secondary antibodies (DakoCytomation California Inc., Carpenteria, CA) and the ECL chemiluminescent system (Amersham Biosciences Inc., Piscataway, NJ) were used for visualization, with exposure times ranging from 1 to 30 s.

Measurement of BaP-DNA Adducts. BaP-DNA adducts were determined by ^{32}P postlabeling as described previously (Talaska et al., 1996). DNA was extracted from liver, small intestine, spleen, or bone marrow using the Wizard genome DNA isolation kit (Promega, Madison, WI) and hydrolyzed (0.5–1.3 μg DNA/sample) to 3′-phosphodeoxynucleotides by digestion with micrococcal endonuclease and spleen phosphodiesterase (both from Sigma-Aldrich). After *n*-butanol extraction to remove most of the nonadducted 3′-phosphodeoxynucleotides, the 3′-phosphodeoxynucleosides were labeled at the 5′ positions with [^{32}P]ATP (PerkinElmer Life and Analytical Sciences, Boston, MA) and T4 polynucleotide kinase (USB, Cleveland, OH). Two-dimensional thin-layer chromatography on polyethylenimine cellulose sheets were used to resolve the ^{32}P-labeled DNA adducts (EM Sciences, Gibbstown, NJ). Visualization and analysis were done by scintillation counting (1900 CA; PerkinElmer Life and Analytical Sciences) and autoradiography, respectively. Adduct levels in each DNA sample were calculated from the background-corrected adduct counts and the specific activity of the radiolabeled-ATP and expressed as relative adduct labeling. The relative adduct labeling is defined as the minimal estimate of the number of nucleotides containing adducts per 10^9 nucleotides in the sample; this value is based on the amount of DNA in each sample and the specific radioactivity of the [^{32}P]ATP used in the assay. Duplicates or triplicates of each DNA sample were analyzed independently.

Statistical Analysis. For quantitative histology, means and S.E.M.s were generated with the General Linear Model (SAS Institute, Cary, NC). For adducts and all other measurements, assays were performed in duplicate or triplicate, and the average values considered as one independent determination. Statistical differences between group mean values were determined by ANOVA and/or Student's pair-wise *t* tests. The data were normally distributed and are presented as means ± S.E.M. A P value < 0.05 was regarded as statistically significant.

Results

CYP1A1 Protects against Oral BaP-Induced Lethality. Previous studies from this laboratory determined a dosing regimen for BaP, in which BaP is delivered to mice in daily gradual doses by ingesting food pellets soaked in corn oil in which BaP had been dissolved (Robinson et al., 1975). Studies using this dosing regimen demonstrated that C57BL/6 mice thrived for at least 1 year with no overt effects of toxicity consuming BaP at 125 mg/kg/day. For this reason, we chose this treatment regimen and dose to compare oral BaP-mediated toxicity between *Cyp1a1*(+/+) and *Cyp1a1*(−/−) mice, both genotypes of which are on a C57BL/6J genetic background. As shown in Fig. 1, the difference between genotypes was very dramatic; oral BaP at this dose was lethal to all *Cyp1a1*(−/−) mice within 30 days, whereas all *Cyp1a1*(+/+) mice, as expected, survived with no overt sign of toxicity.

On the 125 mg/kg/day regimen, food consumption during the first 10 days was not significantly different between *Cyp1a1*(−/−) (3.7 + 1.4 g/day) and *Cyp1a1*(+/+) (4.1 + 1.6 g/day) mice; beyond 10 days, this became significantly ($P < 0.05$) different with the knockout mice consuming less than wild-type mice. No differences in food consumption between *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice were seen at any time on the 12.5 or 1.25 mg/kg/day regimens.

CYP1A1 Drastically Decreases Circulating Blood BaP Levels. Oral BaP (125 mg/kg/day) is strikingly more toxic to *Cyp1a1*(−/−) mice than to *Cyp1a1*(+/+) mice. As a consequence, we initiated additional studies to examine BaP toxicity, spanning two orders of magnitude by including oral BaP dosing regimens of 12.5 and 1.25 mg/kg/day. To understand better the reason why *Cyp1a1*(−/−) mice are susceptible to oral BaP, we evaluated the accumulation of BaP in blood after a gavage dose of BaP (15 mg/kg), chosen roughly to approximate the amount of BaP consumed as a result of a single feeding of BaP at 125 mg/kg/day. When BaP in whole blood was compared in oral BaP-treated *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice (Table 1; Fig. 2), we found the AUC was 4 times greater, and the clearance rate 4 times slower, in *Cyp1a1*(−/−) than in *Cyp1a1*(+/+) mice. The half-life was twice as long in *Cyp1a1*(−/−) than in *Cyp1a1*(+/+) mice.

Although these differences in BaP clearance are quite dramatic, they probably do not reflect those in mice consuming BaP. In untreated mice, CYP1A1 levels are extremely low, approaching undetectable, but are induced by BaP. Thus, to

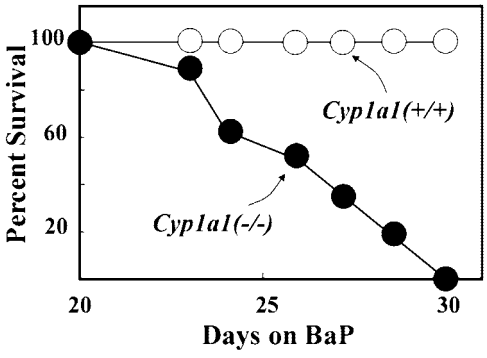

Fig. 1. Comparison of rates of survival between *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice receiving oral BaP (125 mg/kg/day). $n = 6$ per group; no differences were seen between three male mice and three female mice.

study the effect of induced CYP1A1 on circulating BaP, we pretreated mice with TCDD (15 μg/kg), which is known to maximally induce *Cyp1* gene expression without overt toxicity (Nebert, 1989). After TCDD pretreatment (Table 1; Fig. 2), we found the AUC was enhanced to 7 times greater, and the clearance rate 7 times slower, in *Cyp1a1*(−/−) than in *Cyp1a1*(+/+) mice. The half-life was also increased to 4 times as long in *Cyp1a1*(−/−) than in *Cyp1a1*(+/+) mice. The fact that the half-life was the same in untreated as in TCDD-pretreated *Cyp1a1*(−/−) mice (61 versus 63 min) and the fact that TCDD pretreatment decreased the BaP half-life by half in *Cyp1a1*(+/+) mice (31 versus 16 min) are strong evidence that BaP clearance is almost exclusively dependent on inducible CYP1A1 and not other TCDD-inducible BaP-metabolizing phase I enzymes such as CYP1A2 or CYP1B1.

These pharmacokinetics experiments (Table 1, top and middle) were carried out from 15 min to as long as 15 h after BaP gavage into the stomach. To verify that the conclusions drawn from these experiments were practicably reflected in the levels of circulating BaP that resulted from our feeding regimens, we measured BaP in blood 10, 11, and 12 days after initiation of the 125, 12.5, or 1.25 mg/kg/day BaP feeding regimen (Table 1, bottom). BaP was measured on consecutive days to determine whether BaP levels had stabilized or were continuing to rise. In the case of the 125 and 12.5 mg/kg/day dosing regimens, BaP levels were unchanged over the 3-day period, and the data in Table 1 are reported for day 12. In the case of the 1.25 mg/kg/day dosing regimen, BaP levels rose significantly, though not dramatically, during the 3 days (data not shown), and the measurements on day 12 are reported. There was more than an 18-fold greater level of BaP in *Cyp1a1*(−/−) than *Cyp1a1*(+/+) mice at the largest daily oral dose. BaP levels in the blood were too low to detect in *Cyp1a1*(+/+) mice receiving the intermediate and low doses of the chemical. These data confirm that a much greater amount of BaP is available in the blood and distal tissues for longer periods of time in the knockout mouse, compared with that in the wild-type mouse.

Toxicity in the Immune System. *Cyp1a1*(−/−) mice began to appear sickly at about 18 days on oral BaP at the dose of 125 mg/kg/day, but no deaths occurred until 22 days and beyond (Fig. 1). Therefore, we chose 18 days of oral BaP for most of the remaining studies. The immune system was the main target of toxicity, and little evidence of tissue damage was found in other tissues examined. Table 2 shows that *Cyp1a1*(−/−) mice lost weight during these 18 days of oral BaP, compared with BaP-treated *Cyp1a1*(+/+) and control mice of both genotypes, all of which gained weight. Similarly, there was a profound decrease in spleen and thymus weights in the BaP-treated *Cyp1a1*(−/−) mouse, compared with that in the BaP-treated *Cyp1a1*(+/+) and the two control groups. It is probable that this degree of immunotoxicity played a major role in the oral BaP-induced lethality.

Compared with mice on control diet, the spleen weights and thymus weights were also decreased in the *Cyp1a1*(−/−) mouse at the 12.5 mg/kg/day dose of BaP for 18 days. Compared with mice on control diet, the total body weights were also decreased in the *Cyp1a1*(−/−) mouse, even at the 12.5 and 1.25 mg/kg/day doses of BaP for 18 days (Table 2). Therefore, some slight degree of wasting is evident, even at this lowest BaP dose.

Effects of Oral BaP on Plasma AST and ALT Levels. Table 3 shows that plasma AST and ALT levels are markedly elevated in *Cyp1a1*(−/−) mice receiving BaP at the dosage of 125 mg/kg/day, compared with the other three groups. The AST/ALT ratio is often considered to be proportional to the extrahepatic damage/hepatic damage ratio because AST is more specific for extrahepatic tissues and ALT is more specific for liver (Fontana and Lok, 2002). At the 125 mg/kg/day dose, the AST/ALT ratio was 6.3 for BaP-treated *Cyp1a1*(−/−) and 4.9 for *Cyp1a1*(+/+), compared with control ratios of ~2.0. At the 12.5 mg/kg/day dose, the AST/ALT ratio in *Cyp1a1*(−/−) mice was still elevated at 3.0 (Table 4), whereas all other groups exhibited normal AST/ALT ratios (~2.0) at the 12.5 mg/kg/day dose. No differences in AST and ALT levels were seen at the 1.25 mg/kg/day dose of oral BaP (data not shown). Thus, damage to non-hepatic tissues seemed to be more severe than damage to liver in *Cyp1a1*(−/−) at the 125 and 12.5 mg/kg/day BaP doses. These results are consistent with the lack of weight gain in these animals, as shown in Table 2.

Effects of Oral BaP on Peripheral Blood Cells. BaP-treated (125 mg/kg/day) *Cyp1a1*(−/−) mice had markedly lower hemoglobin and hematocrit, higher methemoglobin levels and percent neutrophils, and a very low total lymphocyte count, compared with BaP-treated *Cyp1a1*(+/+) or control mice of either genotype (Table 3). For reasons not clear, the BaP-treated *Cyp1a1*(+/+) mouse exhibited a significantly increased total hemoglobin and decreased methemoglobin levels, compared with control mice. It should be noted that the white cell percentages reported in Table 3 reflect differential counts (i.e., percentages based only on the rela-

TABLE 1

Comparison of BaP pharmacokinetic parameters in *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice

Individual mice (n = 3) were repeatedly tested at 10 time-points for *Cyp1a1*(−/−) and nine time-points for *Cyp1a1*(+/+) mice, following stomach gavage of BaP in corn oil at 15 mg/kg. For blood BaP levels in mice receiving BaP in the food, n was between 6 and 8 animals per group. Values are given as means ± S.D.

	Cyp1a1(−/−)	*Cyp1a1*(+/+)
No TCDD pretreatment, BaP by gavage		
AUC (min/g/ml)	121 ± 8	32 ± 9
Clearance (ml/min/kg)	123 ± 10	490 ± 134
Half-life (min)	61 ± 13	31 ± 11
TCDD pretreated, then BaP gavage		
AUC (min/g/ml)	119 ± 34	16 ± 5
Clearance (ml/min/kg)	135 ± 40	984 ± 310
Half-life (min)	63 ± 10	16 ± 3
BaP concentration (ng/ml) in whole blood after BaP in the food		
BaP, 125 mg/kg/day × 12 days	103 ± 4.0	5.51 ± 2.1
BaP, 12.5 mg/kg/day × 12 days	3.68 ± 2.3	<0.20
BaP, 1.25 mg/kg/day × 12 days	1.20 ± 0.7	<0.20

tive white cell population). In light of this, myeloid lineage cells were most resistant to BaP-induced depletion, and lymphocyte lineage cells were most sensitive. The dramatic drop in the ratio of white to red cells, however, reflects the dramatic loss of the white cell population, despite a large fall in the hematocrit. The increases in nuclear hypersegmentation seen in BaP-treated *Cyp1a1*(−/−) mice, consistent with the anemia, further reflects the leukocytopenia.

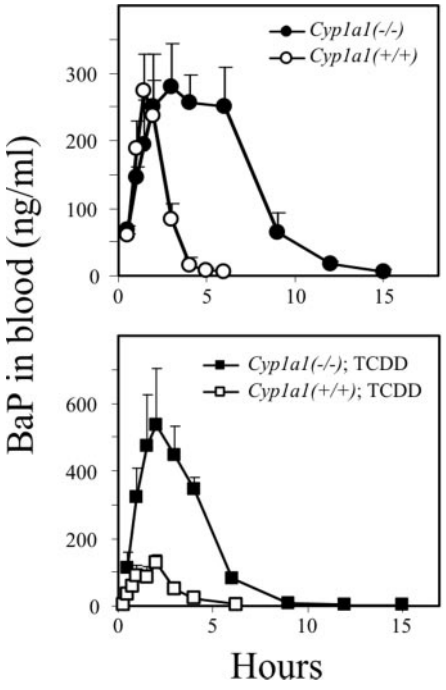

Fig. 2. Comparison of BaP clearance between *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice after stomach gavage of BaP in corn oil at 15 mg/kg. A, without TCDD pretreatment. B, with TCDD pretreatment (15 μg/kg body weight; in corn oil vehicle 48 h before BaP). There were between six and eight animals per group. Values are given as means ± S.D.

The leukocytopenia was not apparent in *Cyp1a1*(−/−) mice receiving oral BaP doses of 12.5 mg/kg/day for 18 days (Table 4), nor were there any differences in peripheral blood among the four groups detectable at 1.25 mg/kg/day for 18 days (data not shown).

Histology. Because of the 2.5-fold elevated plasma ALT activity in *Cyp1a1*(−/−) mice (Table 3), we examined by light microscopy the livers from these four groups of mice. No significant morphological differences were apparent, comparing livers from either genotype control or with either genotype receiving oral BaP at the 125 mg/kg/day dose for 18 days (data not shown). Small intestine and spleen were also examined by light microscopy, and no morphological differences between genotypes of mice receiving oral BaP at 125 mg/kg/day for 18 days were evident (data not shown); the thymus was so atrophied, however, that it was difficult to assess histologically. Intraepithelial lymphocytes in the jejunal villi were within normal limits in all four groups; this finding is in contrast to intraepithelial immune cells (Langerhans and Thy-1 cells) that are known to become significantly depressed in mouse skin after topical application of BaP (Ruby et al., 1989).

Effects of Oral BaP on Bone Marrow. The striking decreases in size of spleen and thymus, coupled with the leukocytopenia, in BaP-treated *Cyp1a1*(−/−) mice led us to suspect that differences would be apparent in the bone marrow. Figure 3 shows that, compared with normal marrow cellularity of the control *Cyp1a1*(−/−) and the BaP-treated

TABLE 2

Comparison of oral BaP for 18 days: effects on body weight and spleen and thymus weights in *Cyp1a1*(−/−) versus *Cyp1a1*(+/+) mice

Values in all tables are expressed as means + S.E.M. Note that the same group of control animals was used for comparison with the three BaP doses. Both BaP-treated groups contained 12 mice; both control groups contained six mice. Any comparison with a P value > 0.05 is not denoted.

	Cyp1a1(−/−)		*Cyp1a1*(+/+)	
	BaP	Control	BaP	Control
Oral BaP, 125 mg/kg/day				
Body weight gain or loss (g)	−5.2 ± 0.2 $P < 0.0001^{a,b,c}$	3.4 ± 0.5	2.4 ± 0.3	2.6 ± 0.4
Spleen weight (mg/g body wt.)	0.5 ± 0.1 $P < 0.0001^{a,b,c}$	3.5 ± 0.1	2.6 ± 0.1 $P = 0.002^{b}$	3.0 ± 0.2
Thymus weight (mg/g body wt.)	0.3 ± 0.0 $P < 0.0001^{a,b,c}$	3.3 ± 0.3	2.0 ± 0.2 $P = 0.026^{a}$ $P = 0.007^{b}$	2.8 ± 0.2
Oral BaP, 12.5 mg/kg/day				
Body weight gain or loss (g)	0.7 ± 0.5 $P = 0.003^{a}$ $P = 0.011^{b}$ $P = 0.0026^{c}$	3.4 ± 0.5	2.6 ± 0.3	2.6 ± 0.4
Spleen weight (mg/g body wt.)	2.4 ± 0.2 $P = 0.001^{a}$	3.5 ± 0.1	2.6 ± 0.1	3.0 ± 0.3
Thymus weight (mg/g body wt.)	1.6 ± 0.3 $P = 0.012^{a}$ $P = 0.034^{b}$ $P = 0.02^{c}$	3.3 ± 0.3	3.0 ± 0.4	2.8 ± 0.2
Oral BaP, 1.25 mg/kg/day				
Body weight gain or loss (g)	1.2 ± 0.5 $P = 0.007^{a}$ $P = 0.034^{b}$ $P = 0.03^{c}$	3.4 ± 0.5	2.7 ± 0.3	2.6 ± 0.4
Spleen weight (mg/g body wt.)	3.1 ± 0.2	3.5 ± 0.1	3.1 ± 0.1	3.0 ± 0.2
Thymus weight (mg/g body wt.)	2.9 ± 0.3	3.3 ± 0.3	2.8 ± 0.5	2.8 ± 0.2

[a] Comparison with control of same genotype.
[b] Comparison with control of other genotype.
[c] Comparison with other BaP-treated group.

Cyp1a1(+/+) mouse, there was an extreme hypocellularity of the marrow in the BaP-treated *Cyp1a1*(−/−) mouse.

As with blood, all nucleated cells of the bone marrow were drastically depleted, as shown in Fig. 3. Relative quantification of the erythroid and myeloid precursors in mice receiving the largest daily oral BaP dose is presented in Table 5. In BaP-treated *Cyp1a1*(−/−) mice, the lymphocyte series of cells was depleted, relative to the myeloid series, a result consistent with circulating white cells (see above). Moreover, the erythroid series of cells was depleted, which is consistent with a decreased hematocrit (see above). In addition, the elevated levels of bone marrow Howell-Jolly bodies, plus increased nuclear hypersegmentation—seen in BaP-treated *Cyp1a1*(−/−) mice, compared with the other three groups (Table 5)—are also consistent with the lowered hematocrit and hemoglobin in these mice (Table 3).

In BaP-treated *Cyp1a1*(−/−) mice receiving the 12.5 mg/kg/day BaP dose (Table 6), there were also significant changes in the relative percentages of white and red cell precursors. None of the alterations in marrow parameters

TABLE 3

Comparison of 125 mg/kg/day × 18 days oral BaP effects: peripheral blood parameters in *Cyp1a1*(−/−) versus *Cyp1a1*(+/+) mice

Both BaP-treated groups have $n = 12$; both control groups have $n = 6$. Any comparison with a P value > 0.05 is not denoted.

	Cyp1a1(−/−)		*Cyp1a1*(+/+)	
	BaP	Control	BaP	Control
AST (units/l)	421 ± 58 $P < 0.0001^{a,b,c}$	54.4 ± 4.0	191 ± 26 $P = 0.015^{a}$ $P = 0.006^{b}$	68.5 ± 8.9
ALT (units/l)	66.5 ± 14 $P = 0.011^{a}$ $P = 0.036^{b}$	27.2 ± 1.5	38.9 ± 6.0	32.3 ± 1.7
Total hemoglobin (mM)	4.8 ± 0.4 $P = 0.022^{a}$ $P = 0.008^{b}$ $P < 0.0001^{c}$	6.3 ± 0.2	10.2 ± 0.1 $P < 0.0001^{a,b}$	6.6 ± 0.1
Methemoglobin (%)	5.2 ± 0.7 $P = 0.042^{a}$ $P = 0.022^{b}$ $P = 0.0007^{c}$	2.9 ± 0.3	1.2 ± 0.2 $P = 0.0004^{a}$ $P = 0.0003^{b}$	2.5 ± 0.2
Hematocrit (%)	25.5 ± 3.3 $P < 0.0001^{a,b,c}$	49.2 ± 0.6	52.5 ± 0.3 $P < 0.0001^{a,b}$	49.3 ± 0.5
Neutrophils (%)	54.2 ± 8.17 $P = 0.0005^{a}$ $P = 0.0002^{b}$ $P < 0.0001^{c}$	18.2 ± 1.2	16.8 ± 2.8	15.2 ± 1.8
Total lymphocytes (%)	29.8 ± 0.8 $P = 0.001^{a}$ $P = 0.0025^{b}$ $P<0.0001^{c}$	66.2 ± 8.3	76.6 ± 3.2	68.5 ± 4.0
Total myeloid (%)	57.9 ± 8.7 $P < 0.0001^{a}$ $P = 0.0025^{b}$ $P < 0.0001^{c}$	22.4 ± 5.8	20.9 ± 2.9	25.6 ± 2.7
Nuclear hypersegmentation (%)	2.3 ± 0.13 $P < 0.0001^{a,b,c}$	1.0 ± 0.3	1.5 ± 0.17	0.5 ± 0.15
White cell-to-red cell ratio	0.05 ± 0.01 $P = 0.0008^{a}$ $P = 0.02^{b}$ $P = 0.02^{c}$	0.44 ± 0.17	0.25 ± 0.04	0.29 ± 0.09

[a] Comparison with control of same genotype.
[b] Comparison with control of other genotype.
[c] Comparison with other BaP-treated group.

TABLE 4

Comparison of 12.5 mg/kg/day × 18 days oral BaP effects: peripheral blood parameters in *Cyp1a1*(−/−) versus *Cyp1a1*(+/+) mice

Each group contains six mice. Note the same control values in this table are also listed in the previous table.

	Cyp1a1(−/−)		*Cyp1a1*(+/+)	
	BaP	Control	BaP	Control
AST (units/l)	87.4 ± 13 $P = 0.035^{a}$ $P = 0.019^{c}$	54.4 ± 4.0	46.3 ± 2.2 $P = 0.026^{a}$	68.8 ± 7.9
ALT (units/l)	29.2 ± 2.5	27.2 ± 1.5	25.0 ± 1.1	32.3 ± 1.7
Total hemoglobin (mM)	7.9 ± 0.1	6.3 ± 0.2	8.3 ± 0.1	6.6 ± 0.1
Hematocrit (%)	49.7 ± 1.0	49.2 ± 0.6	54.3 ± 0.6	49.3 ± 0.5
Neutrophils (%)	20.9 ± 3.2	18.2 ± 5.8	14.3 ± 1.8	15.2 ± 1.8
Total lymphocytes (%)	75.3 ± 2.9	66.2 ± 8.3	82.5 ± 1.9	68.5 ± 4.0
Total myeloid (%)	23.8 ± 2.9	22.4 ± 5.8	15.9 ± 2.0	25.6 ± 2.7

[a] Comparison with control of same genotype.
[b] Comparison with control of other genotype.
[c] Comparison with other BaP-treated group.

was apparent at the 1.25 mg/kg/day dose of BaP for 18 days (data not shown).

Western Immunoblot Analysis. BaP is known to induce several CYP enzymes, in addition to CYP1A1, including CYP1A2 (Landi et al., 1999) and CYP1B1 (Spink et al., 2002). BaP is known to be metabolized to oxygenated reactive intermediates, including the highly toxic and ultimate carcinogen 7,8-dihydroxy-9,10-epoxide, by CYP1A1, CYP1B1 (Yamazaki et al., 2000; Shimada et al., 2001), PTGS2 (Marnett et al., 1982), and perhaps to a small degree by CYP1A2 (Yamazaki et al., 2000). Therefore, we administered oral BaP (125 mg/kg/day) for 5 days and examined the protein levels of these four enzymes in the four tissues most relevant to this study (Fig. 4). CYP1A1 was induced in liver and small intestine of BaP-treated *Cyp1a1*(+/+) mice but not detected in spleen and marrow; as expected, no CYP1A1 was detected in BaP-treated or control *Cyp1a1*(−/−) mice. Basal levels of liver CYP1A2 were seen in control *Cyp1a1*(+/+) and *Cyp1a1*(−/−) mice, and BaP-induced CYP1A2 in both genotypes was found in small intestine but not spleen or marrow. CYP1B1 levels were detectable in the liver of BaP-treated *Cyp1a1*(−/−) mice, more so than *Cyp1a1*(+/+) mice, but found at very low levels in the spleen and not detected in marrow. Basal and BaP-induced levels of CYP1B1 were found in small intestine of both genotypes. PTGS2 was found in liver, small intestine, spleen, and marrow—in both control and BaP-treated mice of both genotypes—and no induction of PTGS2 by BaP was evident. Taken together, these data sug-

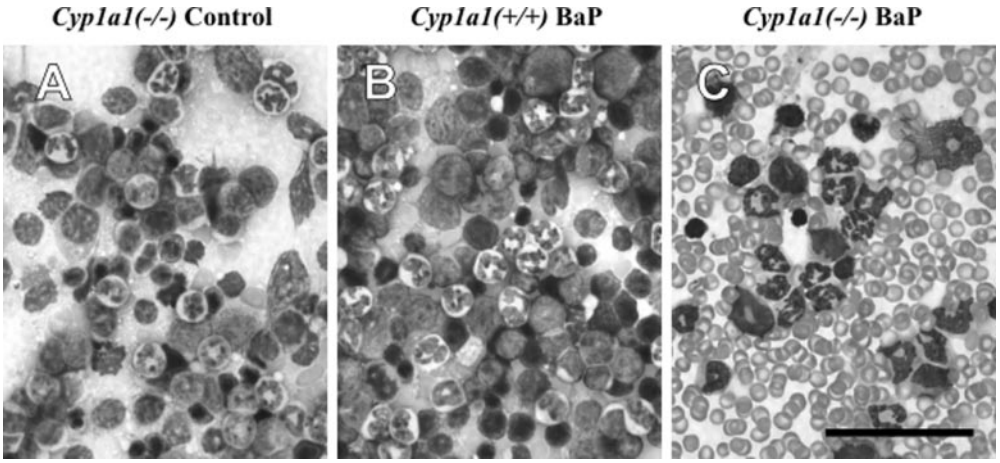

Fig. 3. Histology of bone marrow, comparing p.o. BaP effects on *Cyp1a1*(−/−) versus *Cyp1a1*(+/+) mice. Oral BaP treatment was 125 mg/kg/day for 18 days. A, *Cyp1a1*(−/−) on control diet. B, *Cyp1a1*(+/+) on BaP diet. C, *Cyp1a1*(−/−) on BaP diet, showing severe hypoplasia. Bar = 20 μm.

TABLE 5

Comparison of 125 mg/kg/day × 18 days oral BaP effects on bone marrow parameters of *Cyp1a1*(−/−) versus *Cyp1a1*(+/+) mice

Both BaP-treated groups contain 12 mice; both control groups contain six mice. Any comparison with a P value > 0.05 is not denoted.

	Cyp1a1(−/−)		*Cyp1a1*(+/+)	
	BaP	Control	BaP	Control
		%		
Neutrophils	60.6 ± 9.0 $P = 0.0031^a$ $P = 0.014^b$ $P < 0.0001^c$	29.3 ± 5.2	25.5 ± 1.5	26.4 ± 3.1
Eosinophils	0.5 ± 0.1 $P = 0.0072^a$ $P = 0.0015^b$ $P = 0.0006^c$	2.6 ± 0.3	2.8 ± 0.4	3.0 ± 1.0
Total lymphocytes	6.9 ± 2.3 $P = 0.043^a$ $P = 0.027^b$ $P < 0.0001^c$	14.2 ± 2.5	24.3 ± 1.5 $P = 0.012^a$	15.0 ± 3.3
Total erythroid	17.8 ± 6.6 $P = 0.0099^a$ $P = 0.0077^b$ $P = 0.027^c$	37.6 ± 2.6	31.4 ± 1.6	38.3 ± 2.6
Total myeloid	73.2 ± 7.8 $P = 0.0019^a$ $P = 0.0011^b$ $P < 0.0001^c$	43.7 ± 4.3	38.1 ± 1.9	42.0 ± 3.6
Howell-Jolly bodies	3.2 ± 1.1 $P = 0.01^a$ $P = 0.029^b$ $P = 0.0036^c$	0.13 ± 0.13 $P = 0.0008^a$	0.29 ± 0.1	0.63 ± 0.2
Nuclear hypersegmentation	2.6 ± 0.09 $P = 0.01^a$ $P < 0.0001^{b,c}$	1.8 ± 0.16	1.48 ± 0.21 $P = 0.008^a$	0.66 ± 0.13

[a] Comparison with control of same genotype.
[b] Comparison with control of other genotype.
[c] Comparison with other BaP-treated group.

gest that: 1) CYP1A1 is not present in *Cyp1a1*(−/−) mice, and 2) loss of CYP1A1 does not alter the accumulation of CYP1A2, CYP1B1 or PTGS2 in tissues where these proteins are detected. However, the absence of CYP1B1 accumulation in bone marrow is contrary to other published reports (Heidel et al., 1999; Galvan et al., 2003) and probably represents the relative level of this protein that can be detected by this antibody in S9 fractions (Kerzee and Ramos, 2001; Zhang et al., 2003).

BaP-DNA Adducts. The obvious importance and relevance of an association between BaP-DNA adducts and BaP-induced CYP1A1-mediated toxicity, mutagenesis, teratogenesis, and cancer are well known (Pelkonen and Nebert, 1982; Nebert, 1989; Conney et al., 1994; Knize et al., 1999; Miller and Ramos, 2001). Contrary to what was expected, Fig. 5 shows that BaP-DNA adducts were much greater in animals having no CYP1A1 than in those having BaP-induced CYP1A1. BaP-DNA adducts were dramatically higher in the liver, spleen, and marrow at the 125 or 12.5 mg/kg/day oral dosage. In the small intestine, adduct levels in *Cyp1a1*(−/−) and *Cyp1a1*(+/+) did not differ significantly when mice were fed BaP at 125 mg/kg/day but did differ when mice were fed the 12.5 mg/kg/day dose. Given the dramatic difference in the circulating level of BaP between *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice, this may not be surprising because it is probable that the small intestine is extremely active in metabolizing and eliminating BaP. Thus, although small intestine-derived BaP-DNA adducts do not differ between genotypes at the high BaP dose, if they could be normalized to the metabolic flux through this tissue, the BaP-DNA adducts probably would differ.

Figure 6 illustrates the unique patterns of BaP-DNA adducts in liver, small intestine, spleen, and bone marrow from *Cyp1a1*(+/+) and *Cyp1a1*(−/−) mice. In liver, there were between nine and 13 distinct spots in the *Cyp1a1*(−/−) mouse, compared with only five in the *Cyp1a1*(+/+) mouse; for example, whereas spot 4 in *Cyp1a1*(+/+) liver was not seen in *Cyp1a1*(−/−) liver, the other four spots—plus at least five novel spots—were present in *Cyp1a1*(−/−) liver. In small intestine, the number of BaP-DNA adducts resolved by two-dimensional electrophoresis was at least eight, but each genotype had at least two novel spots not present in the other genotype. In spleen and marrow, the *Cyp1a1*(−/−) mouse exhibited greatly increased amounts of BaP-DNA adducts and, again, each genotype had at least two novel spots not present in the other genotype. Mass spectroscopic analysis of some of the unique BaP-DNA adducts (i.e., found in one genotype but not the other) is underway. Elucidation of the

TABLE 6

Comparison of 12.5 mg/kg/day × 18 days oral BaP effects on bone marrow of *Cyp1a1(−/−) versus Cyp1a1*(+/+) mice

Each group contains six mice. Note the same control values in this table are also listed in the previous table.

	Cyp1a1(−/−)		*Cyp1a1*(+/+)	
	BaP	Control	BaP	Control
		%		
Neutrophils	40.2 ± 1.4 $P = 0.04^a$ $P = 0.012^b$ $P = 0.026^c$	29.3 ± 5.2	28.2 ± 3.3	26.4 ± 3.1
Eosinophils	3.29 ± 1.2	2.6 ± 0.3	3.63 ± 0.88	3.0 ± 1.0
Total lymphocytes	18.2 ± 2.7	14.2 ± 2.5	18.9 ± 1.9	15.0 ± 3.3
Total erythroid	27.4 ± 2.7 $P = 0.022^a$ $P = 0.01^b$	37.6 ± 2.6	31.8 ± 3.4	38.3 ± 2.6
Total myeloid	48.5 ± 0.86 $P = 0.01^c$	43.7 ± 4.3	36.0 ± 3.1	42.0 ± 3.6

[a] Comparison with control of same genotype.
[b] Comparison with control of other genotype.
[c] Comparison with other BaP-treated group.

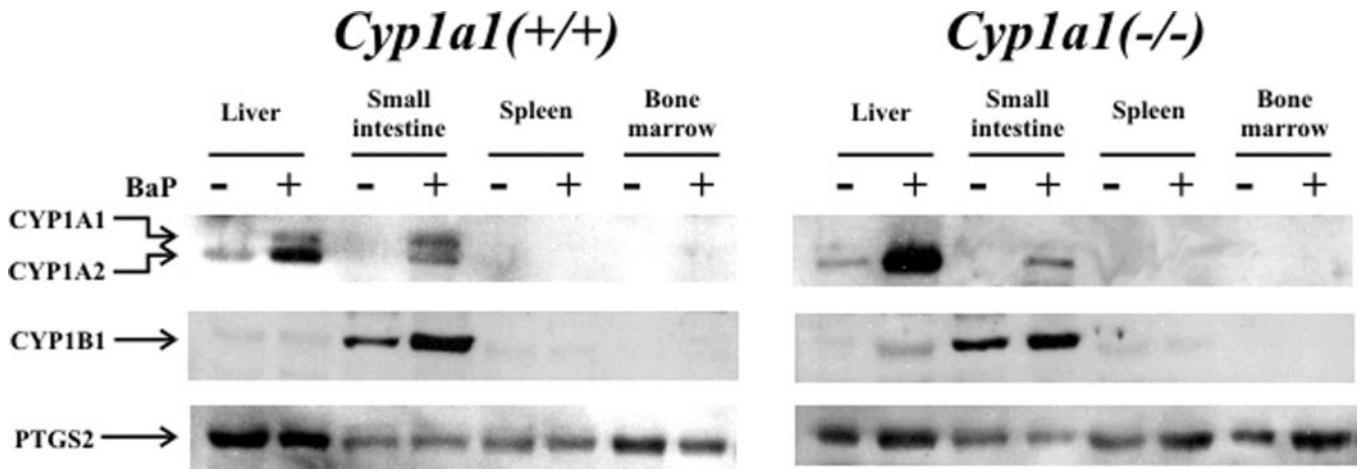

Fig. 4. Western immunoblot of liver, small intestine, spleen, and marrow from control and BaP-treated *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice. Polyclonal antibodies to CYP1A1/1A2, CYP1B1, and PTGS2 were used. Oral BaP treatment was 125 mg/kg/day for 5 days. For CYP1A1/1A2, the lanes were loaded with 2 μg, and for CYP1B1 and PTGS2, the lanes were loaded with 30 μg, of microsomal (S9) protein per lane.

structure of an unknown adduct(s) is not very promising, however, because of differences in sensitivity of the ^{32}P-postlabeling method and mass spectrometry; it is very difficult to accumulate enough material from ^{32}P-postlabeling experiments to achieve BaP-DNA adduct structure elucidation by mass spectrometry.

BaP-DNA adducts derived from the 1.25 mg/kg/day dosing group were too low to quantitate using scintillation counting because our methods for background subtraction were inadequate. Autoradiography of plates developed from these samples, however, do show dramatic increases in BaP-DNA adducts, even in mice receiving the lowest dose of oral BaP for 5 days; Fig. 6B shows the small intestine and spleen data.

***Cyp1a1*(+/+) Mice Are Not Protected against i.p. BaP Administration.** BaP delivered orally must pass through the small intestine and liver before entering the circulation, whereas BaP delivered i.p. is absorbed via the mesenteric veins and lymphatics, and passes through the liver but cannot be metabolized and eliminated immediately by the intestine. To glean some information about the importance of the small intestine in the elimination of BaP, we administered BaP 125 mg/kg/day to *Cyp1a1*(+/+) and *Cyp1a1*(−/−) mice (Fig. 7). Under this regimen, the AUC was 1.8-fold greater in *Cyp1a1*(−/−) than in *Cyp1a1*(+/+) mice, and the half-life was about ~35% longer in *Cyp1a1*(−/−) than in *Cyp1a1*(+/+) mice (data not shown), not nearly as dramatically different as when administered by diet (Table 1). Surprisingly, although lethality occurred with roughly the same timing, there was no significant difference between *Cyp1a1*(+/+) and *Cyp1a1*(−/−) mice (Fig. 7). These data support the notion that intestinal clearance of BaP is ex-

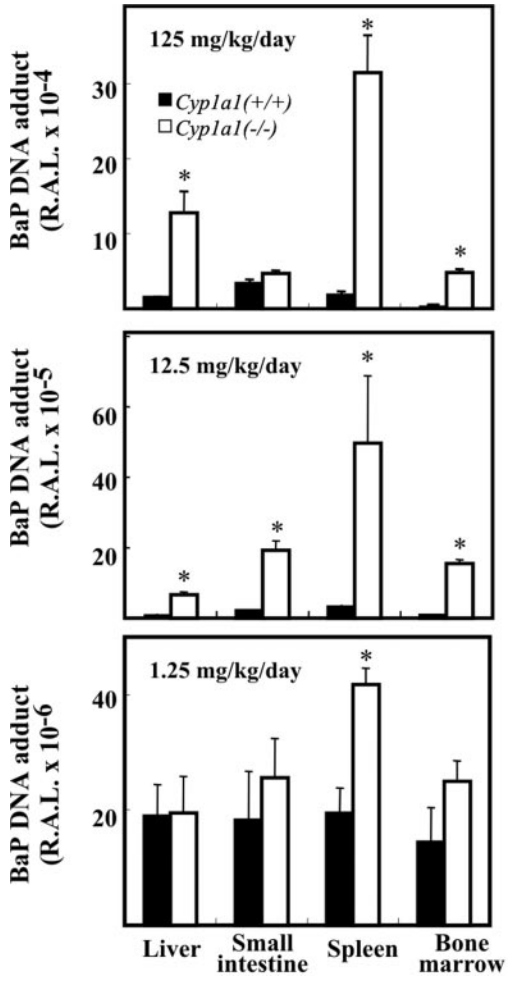

Fig. 5. BaP-DNA adduct formation in liver, small intestine, spleen, and bone marrow from *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice after three oral BaP doses for an 18-day period. Each group had $n = 4$. RAL, relative adduct levels, as detailed under *Materials and Methods*. Note the labeling on the ordinate differs among the three sets of histograms. Bars and brackets denote means ± S.E.M. *, *Cyp1a1*(−/−) value is significantly different ($P < 0.01$) from the *Cyp1a1*(+/+) value in that tissue and at that dose of BaP.

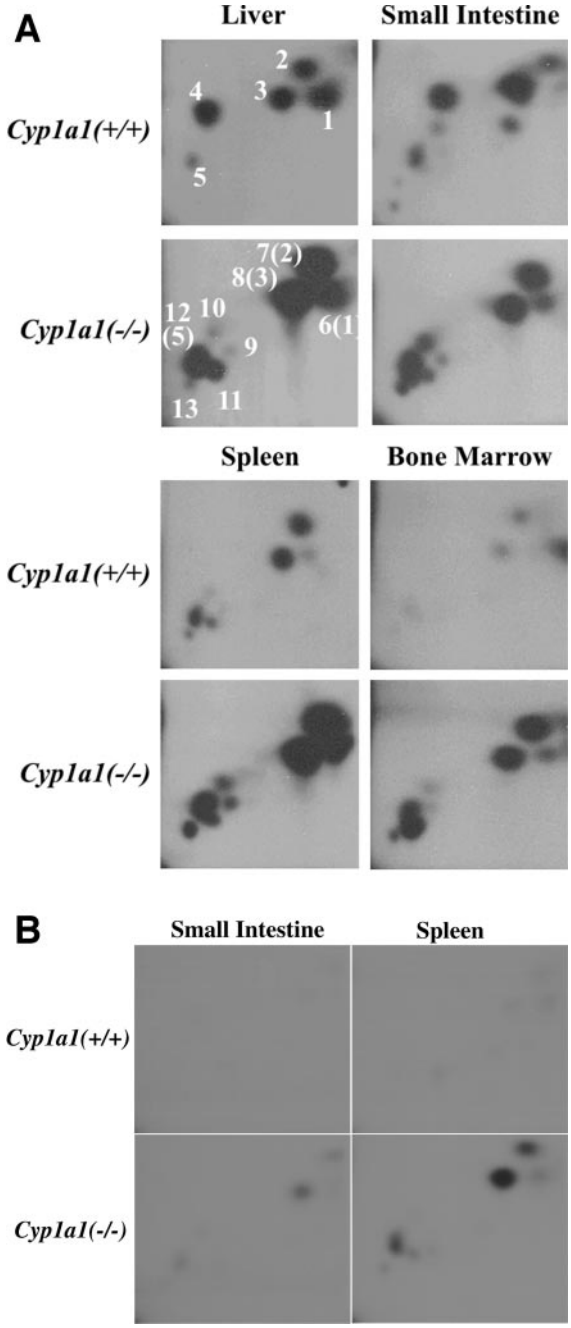

Fig. 6. Autoradiograms after two-dimensional chromatography of the BaP-DNA adducts in: A, liver, small intestine, spleen, and bone marrow from *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice after oral BaP at 125 mg/kg/day for 5 days; and B, in small intestine and spleen from both genotypes after receiving oral BaP at 1.25 mg/kg/day for 5 days. Each is a representative blot of an adduct extract from a tissue that had been tested for BaP-DNA adducts in three individual mice.

tremely important in CYP1A1-mediated protection against oral BaP-induced lethality.

Discussion

In Vitro and Cell Culture Studies: CYP1A1 Is a Determinant of Toxicity. Historically, the role of CYP1A1 in potentiating the toxicity of BaP has found its greatest mechanistic support in studies conducted in the mouse Hepa-1c1c7 hepatoma cell line. The Hepa-1 cell line was derived from a C58/LJ mouse hepatoma (Darlington et al., 1980) and was one of several cell lines derived from this tumor because they retained several differentiated hepatic phenotypes, including albumin synthesis, a trait commonly lost in culture. Hepa-1 cells cultured in the presence of toxic concentrations of BaP grew only rarely as resistant colonies; such colonies were used to complement the resistance phenotype in other colonies, which led to the discovery of at least three complementation groups (Hankinson, 1979; Hankinson, 1995). Eventually, molecular analysis identified the genes representing these complementation groups as *Cyp1a1*, *Ahr*, and *Arnt* encoding the AHR protein's dimerization partner, the aryl hydrocarbon receptor nuclear translocator. Thus, CYP1A1 activates BaP to become toxic, and the AHR and ARNT are necessary for *Cyp1a1* expression. These experiments support the conclusion that CYP1A1 is one of the primary determinants for BaP toxicity. Because these experiments were conducted in hepatoma cells, we postulated that hepatic CYP1A1 would be responsible for BaP-mediated DNA damage in the intact mouse. We were wrong.

Over a range of BaP doses delivered intraperitoneally, hepatic BaP-DNA adducts—which reflect BaP reactive metabolites that are not conjugated and removed—were dramatically higher in *Cyp1a1*(−/−) compared with that in wild-type mice (Uno et al., 2001). Analysis of circulating BaP levels suggested that adducts accumulated to higher levels in *Cyp1a1*(−/−) mice, despite lower rates of hepatic metabolism. Thus, in the context of hepatoma cells, CYP1A1 is the primary determinant of toxicity and DNA adduct formation, whereas in the context of the hepatocyte in the intact animal, CYP1A1 is protective.

In liver, there are several enzymes in addition to CYP1A1—including CYP1B1—that can metabolize BaP. Furthermore, BaP metabolism might be less coupled to phase II elimination enzymes, leading to greater DNA adduct formation. Therefore, the role of a CYP in detoxication versus activation to cause toxicity depends on its subcellular context and its pharmacokinetics. The notion that CYP1A1 is causative in PAH-mediated toxicity and carcinogenesis may not be warranted; in fact, the contrary may be true. These findings underscore the problems 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 cytochromes P450; from this perspective, there can be little doubt that CYP1B1 and CYP1A1 represent major cellular activities toward the metabolism of PAHs. Their roles in causing, preventing, or not participating in PAH-mediated toxicities, however, need further investigation.

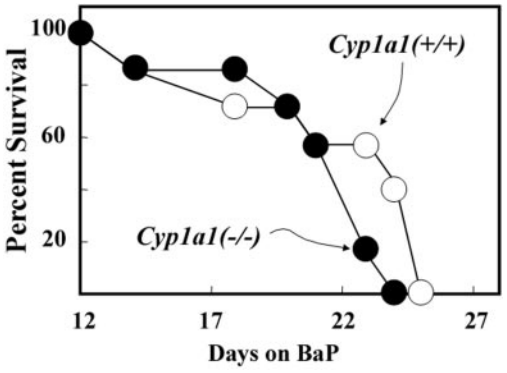

Fig. 7. Comparison of rates of survival between *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice receiving intraperitoneal BaP at the dose of 125 mg/kg/day. $n = 6$ per group; no differences were seen between three male mice and three female mice.

BaP-Induced Immunotoxicity. The oral administration of BaP in *Cyp1a1*(−/−) mice leads to a progressive loss in the mass of immune tissues (Table 2). To determine the extent of these effects, we assessed the effect of BaP (125 mg/kg/day) on cells of the erythroid and myeloid lineage of the bone marrow and blood (Fig. 3; Tables 3 and 5). We conducted these analyses at 4, 8, and 18 days after oral BaP treatment; for the sake of brevity, only the day-18 results on peripheral blood and bone marrow are presented here, although the same trends were evident at 4 and 8 days. The bone marrow of *Cyp1a1*(−/−) mice treated with vehicle alone was within normal limits (Fig. 3), and the marrow of 18-day BaP-treated of *Cyp1a1*(+/+) mice was largely protected against BaP toxicity. The marrow of these groups contained cells of both the erythroid and myeloid lineages. In contrast, after BaP treatment, *Cyp1a1*(−/−) bone marrow smears showed a striking hypocellularity, with loss of both the erythroid and myeloid lineages. The changes in blood (Tables 3 and 4) were reflective of the bone marrow changes (Tables 5 and 6).

Consideration of Other BaP-Activating Enzymes. What enzymes might be responsible for producing far more BaP-DNA adducts in the liver, spleen, and bone marrow of *Cyp1a1*(−/−) mice than wild-type mice (Fig. 5)? Besides CYP1A1 protein differences, we found no important differences between *Cyp1a1*(−/−) and *Cyp1a1*(+/+) mice for CYP1A2, CYP1B1, and PTGS2 in the above-mentioned tissues. One possible exception is the slightly larger amounts of BaP-induced CYP1B1 in *Cyp1a1*(−/−) liver and perhaps spleen (Fig. 4); this observation is under further investigation. Although the anti-CYP1B1 antibody that we used did not detect CYP1B1 in marrow, CYP1B1 metabolism has been detected in marrow (Heidel et al., 1999), and in situ hybridization has recently shown CYP1B1 to be constitutively expressed in marrow (Galvan et al., 2003).

It should also be kept in mind, for possible future studies, that PAHs such as BaP are known to induce one or more forms of cytochrome P450 2A (Kimura et al., 1989), cytochrome P450 2C (Fisslthaler et al., 1999), and cytochrome P450 2S1 (Rivera et al., 2002) enzymes, and that most, if not all, of these enzymes are able to metabolize BaP to oxygenated reactive intermediates.

CYP1B1 in Immune Cells. CYP1B1 is located in brain, prostate, breast, cervix, uterus, ovary, adrenal cortex, and cells of the immune system (Hakkola et al., 1997; Muskhelishvili et al., 2001). High constitutive levels of CYP1B1 exist in bone marrow stroma (Heidel et al., 1998; Galvan et al., 2003) and are necessary for 7,12-dimethylbenzo[*a*]anthra-

cene-induced apoptosis (Heidel et al., 1999). What little CYP1B1 there is in liver is localized to hepatic stellate cells and myofibroblasts, i.e., mesenchymal (Piscaglia et al., 1999), instead of parenchymal, as found for CYP1A2 and inducible CYP1A1 and CYP1A2 (Dey et al., 1999). CYP1B1 is inducible by UV in skin (Katiyar et al., 2000) and also exists in blood monocytes and macrophages (Baron et al., 1998). Before CYP1B1 enzyme activity (metabolic activation of many PAHs) was characterized (Crespi et al., 1997), CYP1A1 was believed to be responsible for virtually all leukocyte BaP hydroxylase activity. CYP1B1 is now known to share with CYP1A1 the PAH-inducible BaP hydroxylase activity in human monocytes, macrophages (Baron et al., 1998; Dassi et al., 1998), lymphocytes (Spencer et al., 1999; Finnstrom et al., 2002; Lin et al., 2003), and bone marrow stromal cells (Heidel et al., 1999; Galvan et al., 2003).

First Pass Elimination Kinetics. Our study points out the important pharmacokinetic differences that depend on the route of administration and the target organ. Although i.p. BaP (125 mg/kg) produced an AUC of 1.8-fold, the same dose of BaP by mouth produced an AUC of 4-fold, comparing *Cyp1a1*(−/−) with *Cyp1a1*(+/+) mice; after 12 days of oral BaP, there was >18-fold difference between the two genotypes (Table 1). Intraperitoneal administration results in the uptake of BaP by mesenteric veins and lymphatics that go directly to the liver, bypassing the gastrointestinal tract, whereas oral administration goes via the gastrointestinal tract and then to the liver. The inducibility of CYP1A1 in the gastrointestinal tract and liver is probably responsible for the much more rapid clearance of oral BaP in wild-type mice, compared with *Cyp1a1*(−/−) mice; this is called first pass elimination kinetics (Routledge and Shand, 1979). It would be interesting to dissect this further by making liver- and gastrointestinal tract-specific *Cyp1a1* knockout mouse lines to see which tissue is more important in generating the large pharmacokinetic differences.

Previous Inbred Mouse Strain Studies on Oral BaP. Previous genetic studies with oral BaP-treated C57BL/6N (B6) mice, having the high-affinity AHR, and DBA/2N (D2) mice, having the poor-affinity AHR (Robinson et al., 1975; Nebert et al., 1980; Legraverend et al., 1983; Nebert, 1989), are similar to what was found in the present study with the knockout mice. The B6 genotype is $Ahr^{b1/b1}$ and the D2 genotype is $Ahr^{d/d}$. On the oral BaP diet of 125 mg/kg/day, *Ahr(d/d)* mice die within 30 days, whereas *Ahr(b1/d)* and *Ahr(b1/b1)* mice remain healthy even after a year at this level of BaP exposure (Robinson et al., 1975). BaP and metabolites, initially for the first 1 to 2 days, can be 20- to 80-fold higher in marrow of *d/d* than *b1/b1* mice, 10- to 20-fold higher in spleen, and ~2-fold higher in small intestine and liver (Nebert et al., 1980). Replacing *d/d* marrow with *b1/b1* marrow and vice versa (Legraverend et al., 1983), it was demonstrated that the oral BaP-induced toxic chemical depression of the marrow and myelotoxicity was always associated with the *d/d* gastrointestinal tract and liver; the genotype of the transplanted marrow did not make any difference in the ultimate endpoint of myelotoxicity. These studies strongly suggested that inducible CYP1A1 in the gastrointestinal tract and liver was able to prevent oral BaP-induced marrow toxicity, simply on the basis of mice having the high- versus poor-affinity AHR (Nebert, 1989). The present study extends these previous studies by examining mice having the genetic absence of CYP1A1 altogether.

Malignancy Instead of Toxicity at Lower BaP Doses. When oral BaP was given at doses of 12 or 6 mg/kg/day for 8 months, the *d/d* mice survived but between 100 and 240 days on this regimen developed leukemias of several types; in contrast, *b1/b1* mice exhibited rates of leukemia no different from controls (Nebert and Jensen, 1979). When a metabolic inhibitor of CYP1A1, α-naphthoflavone, was given orally at 120 mg/kg/day along with the 6 mg of oral BaP, the rate of leukemia formation was greatly decreased, suggesting the involvement of CYP1A1 possibly in the marrow capable of metabolically activating BaP to reactive intermediates (Nebert and Jensen, 1979). Now that CYP1B1 is known to exist in peripheral tissues including bone marrow (Heidel et al., 1998; Galvan et al., 2003) and that CYP1B1 and CYP1A1 have similar substrate specificity profiles with respect to BaP and α-naphthoflavone (Bao et al., 2002), we would like to repeat these leukemia studies in both *Cyp1a1*(−/−) and *Cyp1b1*(−/−) knockout mice.

Conclusion

When BaP is given orally, the absence of CYP1A1 leads to greater disposition of BaP in blood and probably to all distal tissues, and this causes acute toxicity of the cells of the immune system. Does CYP1A2 or CYP1B1 mediate this toxicity? Or does either CYP1A2 or CYP1B1 aid in the detoxication of BaP? Does CYP1A1 directly protect the cells of the immune system?

Inducible CYP1A1 metabolizes BaP to oxygenated reactive intermediates responsible for toxicity, teratogenesis, and carcinogenesis. The common viewpoint on inducible CYP1A1 is that this process is detrimental to humans and lab animals because of the enzyme's capability of metabolic activation. For several decades, pharmaceutical companies have had the general policy to drop from further consideration any test drug that induces CYP1A1 because of its potential risk to cause toxicity and cancer (Valles et al., 1995; Gastel, 2001). It has even been argued that inhibition of CYP1A1, or the genetic disruption of the *CYP1A1* gene, might be beneficial to the animal (or human) by decreasing risk of toxicity or cancer; however, to the contrary, it has been suggested (Nebert and Dieter, 2000) that inducible CYP1A1 probably has a beneficial function to the organism, which is why this gene has been preserved in vertebrates for more than 400 million years.

In contrast to this consensus policy that inducible CYP1A1 is bad for any organism, our study shows just the opposite. Having the *Cyp1a1* gene present and functional, which can lead to striking increases in oral BaP-induced CYP1A1 in the gastrointestinal tract and liver, the *Cyp1a1*(+/+) wild-type mouse is much more protected from immunotoxicity, myelotoxicity, and wasting than the *Cyp1a1*(−/−) knockout mouse. In this paradigm of oral BaP treatment, the inducible CYP1A1 is thus far more important in detoxication than in metabolic activation. Furthermore, delayed clearance in the *Cyp1a1*(−/−) mouse leads to: wasting; immunotoxicity; bone marrow depression; increased BaP-DNA adduct formation in liver, small intestine, spleen, and marrow; and a different spectrum of BaP-DNA adducts being formed in these four tissues (Fig. 6). In future experiments, we will dissect the

importance of inducible CYP1A1, CYP1A2, and CYP1B1 in the liver, intestine, spleen, and marrow.

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References

Alexandrov K, Cascorbi I, Rojas M, Bouvier G, Kriek E, and Bartsch H (2002) *CYP1A1* and *GSTM1* genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis* **23:**1969–1977.

Bao H, Vepakomma M, and Sarkar MA (2002) Benzo[a]pyrene exposure induces CYP1A1 activity and expression in human endometrial cells. *J Steroid Biochem Mol Biol* **81:**37–45.

Baron JM, Zwadlo-Klarwasser G, Jugert F, Hamann W, Rubben A, Mukhtar H, and Merk HF (1998) Cytochrome P450 1B1: a major P450 isoenzyme in human blood monocytes and macrophage subsets. *Biochem Pharmacol* **56:**1105–1110.

Buesen R, Mock M, Seidel A, Jacob J, and Lampen A (2002) Interaction between metabolism and transport of benzo[a]pyrene and its metabolites in enterocytes. *Toxicol Appl Pharmacol* **183:**168–178.

Conney AH, Chang RL, Jerina DM, and Wei SJ (1994) Studies on the metabolism of benzo[a]pyrene and dose-dependent differences in the mutagenic profile of its ultimate carcinogenic metabolite. *Drug Metab Rev* **26:**125–163.

Crespi CL, Penman BW, Steimel DT, Smith T, Yang CS, and Sutter TR (1997) Development of a human lymphoblastoid cell line constitutively expressing human CYP1B1 cDNA: substrate specificity with model substrates and promutagens. *Mutagenesis* **12:**83–89.

Dalton TP, Dieter MZ, Matlib RS, Childs N, Shertzer HG, Genter MB, and Nebert DW (2000) Targeted knockout in the *Cyp1a1* gene does not alter hepatic constitutive expression of other genes in the mouse [*Ah*] battery. *Biochem Biophys Res Commun* **267:**184–189.

Darlington GJ, Bernhard HP, Miller RA, and Ruddle FH (1980) Expression of liver phenotypes in cultured mouse hepatoma cells. *J Natl Cancer Inst* **64:**809–819.

Dassi C, Signorini S, Gerthoux P, Cazzaniga M, and Brambilla P (1998) Cytochrome P450 1B1 mRNA measured in blood mononuclear cells by quantitative reverse transcription-PCR. *Clin Chem* **44:**2416–2421.

Delescluse C, Lemaire G, de Sousa G, and Rahmani R (2000) Is CYP1A1 induction always related to AHR signaling pathway? *Toxicology* **153:**73–82.

Dey A, Jones JE, and Nebert DW (1999) Tissue- and cell type-specific expression of cytochrome P450 1A1 and cytochrome P450 1A2 mRNA in the mouse localized by in situ hybridization. *Biochem Pharmacol* **58:**525–537.

Ellard S, Mohammed Y, Dogra S, Wolfel C, Doehmer J, and Parry JM (1991) Use of genetically engineered V79 Chinese hamster cultures expressing rat liver CYP1A1, 1A2 and 2B1 cDNAs in micronucleus assays. *Mutagenesis* **6:**461–470.

Finnstrom N, Ask B, Dahl ML, Gadd M, and Rane A (2002) Intra-individual variation and sex differences in gene expression of cytochromes P450 in circulating leukocytes. *Pharmacogenomics J* **2:**111–116.

Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, and Busse R (1999) Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature (Lond)* **401:**493–497.

Fontana RJ and Lok AS (2002) Noninvasive monitoring of patients with chronic hepatitis C. *Hepatology* **36 (Suppl 1):**S57–S64.

Galvan N, Jaskula-Sztul R, MacWilliams PS, Czuprynski CJ, and Jefcoate CR (2003) Bone marrow cytotoxicity of benzo[a]pyrene is dependent on CYP1B1 but is diminished by Ah receptor-mediated induction of CYP1A1 in liver. *Toxicol Appl Pharmacol* **193:**84–96.

Garcia-Falcon MS, Gonzalez-Amigo S, Lage-Yusty MA, Lopez de Alda Villaizan MJ, and Simal-Lozano J (1996) Determination of benzo[a]pyrene in lipid-soluble liquid smoke (LSLS) by HPLC-FL. *Food Addit Contam* **13:**863–870.

Gastel JA (2001) Early indicators of response in biologically based risk assessment for nongenotoxic carcinogens. *Regul Toxicol Pharmacol* **33:**393–398.

Hakkola J, Pasanen M, Pelkonen O, Hukkanen J, Evisalmi S, Anttila S, Rane A, Mantyla M, Purkunen R, Saarikoski S, et al. (1997) Expression of CYP1B1 in human adult and fetal tissues and differential inducibility of CYP1B1 and CYP1A1 by Ah receptor ligands in human placenta and cultured cells. *Carcinogenesis* **18:**391–397.

Hankinson O (1979) Single-step selection of clones of a mouse hepatoma line deficient in aryl hydrocarbon hydroxylase. *Proc Natl Acad Sci USA* **76:**373–376.

Hankinson O (1995) The aryl hydrocarbon receptor complex. *Annu Rev Pharmacol Toxicol* **35:**307–340.

Heidel SM, Czuprynski CJ, and Jefcoate CR (1998) Bone marrow stromal cells constitutively express high levels of cytochrome P450 1B1 that metabolize 7,12-dimethylbenzo[*a*]anthracene. *Mol Pharmacol* **54:**1000–1006.

Heidel SM, Holston K, Buters JT, Gonzalez FJ, Jefcoate CR, and Czupyrynski CJ (1999) Bone marrow stromal cell cytochrome P450 1B1 is required for pre-B cell apoptosis induced by 7,12-dimethylbenzo[a]anthracene. *Mol Pharmacol* **56:**1317–1323.

Hem A, Smith AJ, and Solberg P (1998) Saphenous vein puncture for blood sampling of the mouse, rat, hamster, gerbil, guinea pig, ferret and mink. *Lab Anim* **32:**364–368.

Katiyar SK, Matsui MS, and Mukhtar H (2000) Ultraviolet-B exposure of human skin induces cytochromes P450 1A1 and 1B1. *J Investig Dermatol* **114:**328–333.

Kerzee JK and Ramos KS (2001) Constitutive and inducible expression of *Cyp1a1* and *Cyp1b1* in vascular smooth-muscle cells: role of the AHR bHLH/PAS transcription factor. *Circ Res* **89:**573–582.

Kim HS, Kwack SJ, and Lee BM (2000) Lipid peroxidation, antioxidant enzymes and benzo[*a*]pyrene-quinones in the blood of rats treated with benzo[*a*]pyrene. *Chem Biol Interact* **127:**139–150.

Kimura S, Kozak CA, and Gonzalez FJ (1989) Identification of a novel P450 expressed in rat lung: cDNA cloning and sequence, chromosome mapping and induction by 3-methylcholanthrene. *Biochemistry* **28:**3798–3803.

Knize MG, Salmon CP, Pais P, and Felton JS (1999) Food heating and the formation of heterocyclic aromatic amine and polycyclic aromatic hydrocarbon mutagens/carcinogens. *Adv Exp Med Biol* **459:**179–193.

Landi MT, Sinha R, Lang NP, and Kadlubar FF (1999) Human cytochrome P450 1A2. *IARC Sci Publ* **148:**173–195.

Legraverend C, Harrison DE, Ruscetti FW, and Nebert DW (1983) Bone marrow toxicity induced by oral benzo[a]pyrene: protection resides at the level of the intestine and liver. *Toxicol Appl Pharmacol* **70:**390–401.

Lin P, Hu SW, and Chang TH (2003) Correlation between gene expression of aryl hydrocarbon receptor (*AHR*), hydrocarbon receptor nuclear translocator (*ARNT*), cytochromes P450 1A1 (*CYP1A1*) and 1B1 (*CYP1B1*) and inducibility of CYP1A1 and CYP1B1 in human lymphocytes. *Toxicol Sci* **71:**20–26.

Marnett LJ, Panthananickal A, and Reed GA (1982) Metabolic activation of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene during prostaglandin biosynthesis. *Drug Metab Rev* **13:**235–247.

Miller KP and Ramos KS (2001) Impact of cellular metabolism on the biological effects of benzo[a]pyrene and related hydrocarbons. *Drug Metab Rev* **33:**1–35.

Mimura J and Fujii-Kuriyama Y (2003) Functional role of the AHR in the expression of toxic effects by TCDD. *Biochim Biophys Acta* **1619:**263–268.

Muskhelishvili L, Thompson PA, Kusewitt DF, Wang C, and Kadlubar FF (2001) In situ hybridization and immunohistochemical analysis of cytochrome P450 1B1 expression in human normal tissues. *J Histochem Cytochem* **49:**229–236.

Nebert DW (1989) The *Ah* locus: Genetic differences in toxicity, cancer, mutation and birth defects. *Crit Rev Toxicol* **20:**153–174.

Nebert DW, Dalton TP, Stuart GW, and Carvan MJ III (2000a) "Gene-swap knock-in" cassette in mice to study allelic differences in human genes. *Ann NY Acad Sci* **919:**148–170.

Nebert DW and Dieter MZ (2000) The evolution of drug metabolism. *Pharmacology* **61:**124–135.

Nebert DW and Jensen NM (1979) Benzo[a]pyrene-initiated leukemia in mice: association with allelic differences at the *Ah* locus. *Biochem Pharmacol* **27:**149–151.

Nebert DW, Jensen NM, Levitt RC, and Felton JS (1980) Toxic chemical depression of the bone marrow and possible aplastic anemia explainable on a genetic basis. *Clin Toxicol* **16:**99–122.

Nebert DW, Roe AL, Dieter MZ, Solis WA, Yang Y, and Dalton TP (2000b) Role of the aromatic hydrocarbon receptor and [*Ah*] gene battery in the oxidative stress response, cell cycle control and apoptosis. *Biochem Pharmacol* **59:**65–85.

Pelkonen O and Nebert DW (1982) Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. *Pharmacol Rev* **34:**189–222.

Piscaglia F, Knittel T, Kobold D, Barnikol-Watanabe S, Di Rocco P, and Ramadori G (1999) Cellular localization of hepatic cytochrome P450 1B1 expression and its regulation by aromatic hydrocarbons and inflammatory cytokines. *Biochem Pharmacol* **58:**157–165.

Puga A, Maier A, and Medvedovic M (2000) The transcriptional signature of dioxin in human hepatoma HepG2 cells. *Biochem Pharmacol* **60:**1129–1142.

Puga A, Nebert DW, and Carrier F (1992) Dioxin induces expression of c-*fos* and c-*jun* proto-oncogenes and a large increase in transcriptional factor AP-1. *DNA Cell Biol* **11:**269–281.

Rivera SP, Saarikoski ST, and Hankinson O (2002) Identification of a novel dioxin-inducible cytochrome P450. *Mol Pharmacol* **61:**255–259.

Robinson JR, Felton JS, Levitt RC, Thorgeirsson SS, and Nebert DW (1975) Relationship between "aromatic hydrocarbon responsiveness" and the survival times in mice treated with various drugs and environmental compounds. *Mol Pharmacol* **11:**850–865.

Routledge PA and Shand DG (1979) Clinical pharmacokinetics of propranolol. *Clin Pharmacokinet* **4:**73–90.

Ruby JC, Halliday GM, and Muller HK (1989) Differential effects of benzo[a]pyrene and dimethylbenzo[a]anthracene on Langerhans cell distribution and contact sensitization in murine epidermis. *J Investig Dermatol* **92:**150–155.

Ryu DY, Levi PE, Fernandez-Salguero P, Gonzalez FJ, and Hodgson E (1996) Piperonyl butoxide and acenaphthylene induce cytochrome P450 1A2 and 1B1 mRNA in aromatic hydrocarbon-responsive receptor knock-out mouse liver. *Mol Pharmacol* **50:**443–446.

Shimada T, Oda Y, Gillam EM, Guengerich FP, and Inoue K (2001) Metabolic activation of polycyclic aromatic hydrocarbons and other procarcinogens by cytochromes P450 1A1 and P450 1B1 allelic variants and other human cytochromes P450 in *Salmonella typhimurium* NM2009. *Drug Metab Dispos* **29:**1176–1182.

Spencer DL, Masten SA, Lanier KM, Yang X, Grassman JA, Miller CR, Sutter TR, Lucier GW, and Walker NJ (1999) Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* **8:**139–146.

Spink DC, Katz BH, Hussain MM, Spink BC, Wu SJ, Liu N, Pause R, and Kaminsky LS (2002) Induction of CYP1A1 and CYP1B1 in T-47D human breast cancer cells by benzo[*a*]pyrene is diminished by arsenite. *Drug Metab Dispos* **30:**262–269.

Talaska G, Cudnik J, Jaeger M, Rothman N, Hayes R, Bhatnagar VJ, and Kayshup SJ (1996) Development and application of non-invasive biomarkers for carcinogen-DNA adduct analysis in occupationally exposed populations. *Toxicology* **111:**207–212.

Uno S, Dalton TP, Shertzer HG, Genter MB, Warshawsky D, Talaska G, and Nebert DW (2001) Benzo[a]pyrene-induced toxicity: paradoxical protection in *Cyp1a1*(−/−) knockout mice having increased hepatic BaP-DNA adduct levels. *Biochem Biophys Res Commun* **289:**1049–1056.

Valles B, Schiller CD, Coassolo P, De Sousa G, Wyss R, Jaeck D, Viger-Chougnet A, and Rahmani R (1995) Metabolism of mofarotene in hepatocytes and liver micro-

somes from different species. Comparison with in vivo data and evaluation of the cytochrome P450 isoenzymes involved in human biotransformation. *Drug Metab Dispos* **23:**1051–1057.

Walker NJ, Gastel JA, Costa LT, Clark GC, Lucier GW, and Sutter TR (1995) Rat CYP1B1: an adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. *Carcinogenesis* **16:**1319–1327.

Yamazaki H, Hatanaka N, Kizu R, Hayakawa K, Shimada N, Guengerich FP, Nakajima M, and Yokoi T (2000) Bioactivation of diesel exhaust particle extracts and their major nitrated polycyclic aromatic hydrocarbon components, 1-nitropyrene and dinitropyrenes, by human cytochromes P450 1A1, 1A2 and 1B1. *Mutat Res* **472:**129–138.

Zhang QY, Dunbar D, and Kaminsky LS (2003) Characterization of mouse small intestinal cytochrome P450 expression. *Drug Metab Dispos* **31:**1346–1351.

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