The Role of Small-Intestinal P450 Enzymes in Protection against Systemic Exposure of Orally Administered Benzo[a]Pyrene

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ABSTRACT

An intestinal epithelium-specific cytochrome P450 (P450) reductase (CPR)-knockout (IE-Cpr-null) mouse and a liver-specific CPR-knockout (liver-Cpr-null) mouse were studied for determination of the respective roles of P450 enzymes in the liver and small intestine (SI) in the clearance of orally administered benzo[a]pyrene (BaP). Pharmacokinetic analysis of blood BaP levels indicated significantly lower rates of BaP clearance in IE-Cpr-null than in wild-type (WT) mice, after oral BaP (30 mg/kg) treatment. In contrast, clearance rates for intraperitoneal BaP (45 mg/kg) were not different between IE-Cpr-null and WT mice. Furthermore, there was no significant difference between liver-Cpr-null and WT mice in BaP clearance, after either intraperitoneal or oral BaP administration. Thus, small-intestinal P450-mediated first-pass metabolism is a key determinant of

the systemic bioavailability of oral BaP. In addition, we observed greater differences in the rates of clearance of oral BaP, between WT and IE-Cpr-null mice, in mice pretreated with β -naphthoflavone, to induce CYP1A1 expression, than in untreated mice. The onset of induction (at 2 h after dosing) of CYP1A1 protein expression by oral BaP administration was earlier in the SI than in extra-gut organs analyzed; for liver, lung, and kidney, induction was not observed until 4 h after dosing. Furthermore, BaP tissue burdens in SI and extra-gut organs of IE-Cpr-null mice were greater than burdens in corresponding organs of WT mice, at 6 or 24 h after BaP administration. Taken together, these findings strongly support the concept that small-intestinal CYP1A1 induction is a critical factor in protection against systemic exposure to oral BaP.

Benzo[a]pyrene (BaP) is one of the most widespread environmental toxicants and carcinogens. Animal studies indicated that BaP exposure can cause immunotoxicity and tumor formation in multiple organs, such as skin (Shimizu et al., 2000; Uno et al., 2004). BaP may also be one of the compounds responsible for smoking-related human lung cancer, given that BaP DNA adducts have been found to form preferentially in the mutational hot spots of the p53 tumor suppressor gene in human lung cancer (Denissenko et al., 1996). Besides respiratory exposure through inhalation of polluted air, another major route for human BaP exposure is through consumption of BaP-containing foods, for example, overcooked or barbecued meats (Phillips, 1999). Epidemiologic studies indicated that higher BaP intake via daily food consumption is associated with increased risks of colorectal

adenomas in humans (Sinha et al., 2005). BaP-caused toxicities are mediated by the reactive intermediates of the compound, formed during BaP metabolism by the microsomal cytochrome P450 (P450) enzymes, such as CYP1A1 and CYP1B1; the reactive intermediates can bind to DNA, forming covalent adducts, which eventually cause carcinogenesis or other forms of toxicities.

P450-mediated metabolism of BaP is also essential for the detoxification process. In an elegant series of studies that used a combination of single and double P450 gene-knockout mouse models, it was convincingly demonstrated that CYP1A1 is the major enzyme responsible for the detoxification of orally ingested BaP, whereas CYP1B1 is the major enzyme responsible for BaP-induced chemical toxicity (Uno et al., 2004, 2006). Nevertheless, the specific contributions of P450 enzymes in individual organs to overall BaP clearance remained to be determined. In general, the liver, with its abundant P450 enzymes, is the major metabolic organ; loss of hepatic P450 function will, in most cases, lead to reduced rates of clearance of xenobiotics, as has been

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ABBREVIATIONS: BaP, benzo[a]pyrene; P450, cytochrome P450; SI, small intestine; CPR, cytochrome P450 reductase; liver-Cpr-null, liver-specific Cpr-knockout; IE-Cpr-null, intestinal epithelium-specific Cpr-null; WT, wild type; BNF, β -naphthoflavone; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; HPLC, high-performance liquid chromatography; AhR, aryl hydrocarbon receptor; AUC, area under the curve.

demonstrated in mouse models with liver-specific deletion of the NADPH-cytochrome P450 reductase gene (*Cpr* or *Por*) (e.g., Zhang et al., 2007; Fang et al., 2008a). However, for orally ingested xenobiotics, the small intestine (SI) is the portal-of-entry organ; therefore, P450 enzymes in the SI may also play an essential role in the first-pass metabolism of absorbed xenobiotics, as has been demonstrated for nifedipine (Zhang et al., 2007, 2009) and docetaxel (van Herwaarden et al., 2007). In that context, the SI is a known target organ for BaP toxicity (Brooks et al., 1999; Culp et al., 2000), and oral exposure of mice to BaP leads to DNA adduct formation in the SI (Brauze et al., 1991; Uno et al., 2004). Notably, the relative contributions of the liver and the SI to the overall clearance of oral BaP could not be resolved using the available *Cyp1a1*-knockout mouse model (Uno et al., 2004), given that the *Cyp1a1* gene was deleted globally in that model.

CPR is the obligatory electron donor for all microsomal P450 enzymes; deletion of the *Cpr* gene in a given cell will cause the loss of P450 activities in that cell. Several tissue-specific *Cpr*-null mouse models have been generated in recent years (Gu et al., 2003; Henderson et al., 2003; Weng et al., 2007; Fang et al., 2008b; Zhang et al., 2009), for studies on P450 functions in specific organs. Here, we have used a liver-specific *Cpr*-knockout mouse model (liver-*Cpr*-null) (Gu et al., 2003) and an intestinal epithelium-specific *Cpr*-knockout mouse model (IE-*Cpr*-null) (Zhang et al., 2009), to directly evaluate the respective roles of intestinal and hepatic P450s in BaP clearance. Pharmacokinetic analyses of blood BaP levels were performed for IE-*Cpr*-null, liver-*Cpr*-null, and wild-type (WT) mice, after either oral or intraperitoneal BaP administration. Given that the constitutive expression of CYP1A1 is very low in both liver and SI, yet CYP1A1 is highly inducible by xenobiotic inducers, including BaP (Uno et al., 2008), we also compared, between WT and IE-*Cpr*-null mice, the rates of clearance of oral BaP in mice that had been pretreated with β -naphthoflavone (BNF) to induce CYP1A1 expression. Furthermore, we determined the time course of induction of CYP1A1 protein by oral BaP administration in the SI and extra-gut organs of WT mice, in an effort to determine whether the CYP1A1 induction in the SI is rapid enough to cause increased first-pass metabolism of the compound itself, after a single-dose exposure. In addition, BaP tissue burdens in the SI and extra-gut organs of WT and IE-*Cpr*-null mice were compared, at 6 and 24 h after BaP administration, to directly demonstrate the role of small-intestinal P450 enzymes in protection against systemic exposure to oral BaP.

Materials and Methods

Animals and Treatments. Adult (2–3-month-old) IE-*Cpr*-null (Zhang et al., 2009) and liver-*Cpr*-null (Gu et al., 2003) mice (both congenic on the C57BL/6 background) as well as WT littermates or C57BL/6 mice were used. Animals were given food and water ad libitum. For oral gavage, male mice were given a bolus dose of BaP (15 or 30 mg/kg; Sigma-Aldrich, St. Louis, MO), dissolved in dimethyl sulfoxide (DMSO)/corn oil [1:9 (v/v)] at 3 mg/ml. For the intraperitoneal route, male mice were injected with BaP (45 mg/kg), dissolved in DMSO/corn oil [3:7 (v/v)] at 4.5 mg/ml. This vehicle formulation was found in our pilot studies to provide greater BaP bioavailability than that provided by pure corn oil. For CYP1A1 induction studies, mice were injected intraperitoneally, once daily

with 40 mg/kg BNF, dissolved in DMSO/corn oil [1:7 (v/v)] at 4 mg/ml, for 3 consecutive days, before tissues were obtained, or before further treatment with BaP for pharmacokinetics studies. All animal studies were approved by the Institutional Animal Care and Use Committee of the Wadsworth Center (Albany, NY).

Pharmacokinetic Study. Blood samples (50 μ l) were obtained through the tail vein at 0.5, 1, 2, 4, and 6 h after BaP treatment. The samples were diluted with 0.2 ml of phosphate-buffered saline (PBS) and then extracted with use of 1 ml of ethyl acetate. The phases were separated by centrifugation at 1500g for 5 min. The organic layer was collected, and the aqueous layer was extracted again with 1 ml of ethyl acetate. The pooled organic layer was dried under nitrogen. The residue was resuspended in 200 μ l of methanol; a 100- μ l portion was used for HPLC analysis. Recovery of spiked BaP standard was $\geq 85\%$. Samples were analyzed on a Nova-Pak C₁₈ column (5 μ m, 8 $\times$ 100 mm; Waters, Milford, MA) preceded by a C₁₈ precolumn cartridge, with an Alliance model 2690 HPLC system (Waters). BaP was detected with an online fluorescence detector (model 474; Waters), with excitation at 294 nm and emission at 404 nm, according to a protocol described previously (Uno et al., 2004).

BaP Hydroxylase Activity Assay. The rates of formation of 3-hydroxy-BaP from BaP in microsomal reactions were determined according to the method of Nebert and Gelboin (1968). The amounts of 3-hydroxy-BaP were measured with use of an LS50B luminescence spectrometer (PerkinElmer Life and Analytical Sciences, Boston, MA), with excitation at 396 nm and emission at 522 nm. Authentic 3-hydroxy-BaP (Midwest Research Institute, Kansas City, MO) was used to establish standard curves.

Immunoblot Analysis. Microsomal proteins were resolved on 10% NuPAGE Bis-Tris-gels (Invitrogen, Carlsbad, CA) and then transferred to nitrocellulose membranes. For immunodetection, a polyclonal rabbit anti-rat CYP1A1 (Millipore, San Diego, CA), which does not cross-react with CYP1A2, was used. Peroxidase-labeled goat anti-rabbit IgG (Sigma-Aldrich) was detected with an enhanced chemiluminescence kit (GE Healthcare, Little Chalfont, Buckinghamshire, UK), and immunoblot quantification was carried out using a Typhoon 9400 variable mode imager (GE Healthcare).

Time Course Analysis for CYP1A1 Induction. Adult male C57BL/6 WT mice were treated with BaP (15 mg/kg) by oral gavage. At 0, 2, 4, 6, or 24 h after the oral administration, mice were sacrificed, and the SI, liver, lung, and kidney were dissected; liver, lung, and kidney were stored at -80°C until they were used for microsomal preparation, whereas the SI epithelial cells were isolated and used immediately for microsomal preparation.

Determination of BaP Tissue Levels. Male IE-*Cpr*-null and WT mice (3 months old) were treated with BaP at the dose of 30 mg/kg by oral gavage. At 6 h or 24 h after treatment, mice were sacrificed, and the SI (duodenum), colon, kidney, spleen, lung, and thymus were dissected. For the SI and colon, the intestine was first flushed with 10 ml of ice-cold PBS, to remove intestinal contents. The intestine was then cut open lengthwise and rinsed twice in ice-cold PBS. The tissues were then blotted on a paper towel and stored at -80°C until use. BaP was extracted as described by Galván et al. (2005). In brief, the tissues (100–200 mg) were homogenized in 0.1 M Tris-acetate buffer, pH 7.4, containing 1 mM EDTA and 150 mM KCl [1/10 (w/v)], using a Polytron homogenizer (Kinematica, Littau-Lucerne, Switzerland), at a setting of ~ 5000 to 6000 rpm, for 60 s; samples were kept on ice during the homogenization. Tissue homogenate (1 ml) was spiked with an internal standard, dibenzo[*a,h*]pyrene (0.2 μ g, added in 10 μ l of methanol) (Midwest Research Institute), and then mixed with 2 ml of ethyl acetate; the mixture was vortexed vigorously for 5 min, followed by centrifugation at 1500g for 5 min. The organic layer was saved, whereas the aqueous layer was extracted with another 2 ml of ethyl acetate. The organic fractions from the two extractions were combined and then dried under nitrogen. The residue was dissolved in 200 μ l of methanol; any insoluble material was removed by centrifugation, and 100 μ l of the solution were injected for HPLC analysis of BaP.

Other Methods. Isolation of intestinal epithelial cells and preparation of microsomes were performed as reported previously (Zhang et al., 2003). The protein concentration was measured using the bicinchoninic acid method (Pierce Chemical, Rockford, IL). Pharmacokinetic parameters were calculated with the WinNonlin software, version 5.1 (Pharsight, Mountain View, CA). Statistical significance of differences between groups was examined with Student's *t* test.

Results

Small-Intestinal P450, but Not Hepatic P450, Plays an Important Role in Protection against Orally Administered BaP. To directly evaluate the role of small-intestinal and hepatic P450s in the clearance of orally administered BaP, we performed pharmacokinetic studies in liver-Cpr-null, IE-Cpr-null, and WT mice. The pharmacokinetic profiles and parameters are shown in Fig. 1 and Table 1, respec-

tively. When administered orally at 30 mg/kg, BaP was cleared fairly quickly in WT mice and was no longer detected in the blood at 6 h after dosing. However, BaP clearance in the IE-Cpr-null mice was much slower: compared with WT mice, the IE-Cpr-null mice had significantly higher blood BaP levels at 2, 4, and 6 h after dosing (Fig. 1A). The $AUC_{0-6\text{ h}}$, $C_{\max}$, and $t_{1/2}$ values were 3.1-, 1.8-, and 2.1-fold greater, respectively, in IE-Cpr-null than in WT mice (Table 1, experiment 1). In contrast, a significant difference was not found, either in pharmacokinetic profiles (Fig. 1B) or in any of the pharmacokinetic parameters determined (Table 1, experiment 2), between liver-Cpr-null and WT mice. These findings indicate that SI P450, but not hepatic P450, plays an important role in protection against orally administered BaP.

Neither Small-Intestinal P450 nor Hepatic P450 Plays an Important Role in the Clearance of Intraperitoneally Administered BaP. To evaluate the role of small-intestinal and hepatic P450 enzymes in the clearance of intraperitoneally administered BaP, we treated liver-Cpr-null, IE-Cpr-null, and WT mice with a single intraperitoneal injection of BaP at 45 mg/kg. The pharmacokinetic profiles and parameters are shown in Fig. 2 and Table 2, respectively. A significant difference was not found, either in pharmacokinetic profiles, or in any of the pharmacokinetic parameters determined, between IE-Cpr-null and WT mice (Fig. 2A and Table 2, experiment 1). Slight differences in blood BaP levels, at the 2- and the 6-h time points (Fig. 2B), and in the $t_{1/2}$ values (Table 2, experiment 2), were found between the WT and the liver-Cpr-null mice; however, there was no difference in either $C_{\max}$ or AUC values between the two groups. These findings indicate that neither SI P450 nor hepatic P450 is essential for the clearance of intraperitoneally administered BaP, at least at the BaP dosage used.

Role of CYP1A1 Induction in BaP Metabolism and Clearance. CYP1A1 has been shown to be a critical enzyme for BaP metabolism, and CYP1A1 induction is known to increase rates of BaP clearance (Uno et al., 2004). However, the impact of CYP1A1 induction in the SI, on the first-pass clearance of oral BaP, has not been directly demonstrated. Accordingly, we further investigated the clearance of oral BaP in IE-Cpr-null and WT mice that had been pre-exposed to the CYP1A1 inducer BNF.

CYP1A1 induction by BNF in the liver and SI of both IE-Cpr-null and WT mice was demonstrated through immunoblot analysis (Fig. 3A). In agreement with the results of our previous report (Zhang et al., 2009), the level of constitutive expression of CYP1A1 protein in the SI was low in WT mice; however, a striking compensatory increase in CYP1A1 expression was found in the IE-Cpr-null mice. BNF treatment led to increases in SI CYP1A1 expression in both WT and IE-Cpr-null mice. In the liver, CYP1A1 protein was not

Fig. 1. Blood BaP levels in IE-Cpr-null, liver-Cpr-null, and WT mice after a single oral dose of BaP. Adult male mice were given a single oral dose of BaP at 30 mg/kg. Blood samples were collected at various time points after dosing. BaP was detected by HPLC with a fluorescence detector. The IE-Cpr-null (A) and the liver-Cpr-null (B) mice were compared with WT mice in two separate experiments. Values represent means $\pm$ S.D. ($n = 5-6$). *, $P < 0.05$; **, $P < 0.01$, compared with WT mice (Student's *t* test).

TABLE 1

Pharmacokinetic parameters for orally administered BaP in IE-Cpr-null, liver-Cpr-null, and WT mice

Data from Fig. 1 were analyzed for determination of pharmacokinetics parameters. Values represent means $\pm$ S.D. ($n = 5-6$).

Experiment No.	Strain	$T_{\max}$ <i>h</i>	$C_{\max}$ <i>ng/ml</i>	$t_{1/2}$ <i>h</i>	$AUC_{0-6\text{ h}}$ <i>ng · h/ml</i>
1	WT	0.92	215 $\pm$ 72	0.75 $\pm$ 0.16	352 $\pm$ 97
	IE-Cpr-null	1.66	378 $\pm$ 120*	1.57 $\pm$ 0.54**	1090 $\pm$ 410**
2	WT	1.20	234 $\pm$ 88	0.74 $\pm$ 0.31	447 $\pm$ 112
	liver-Cpr-null	1.40	251 $\pm$ 143	0.84 $\pm$ 0.32	618 $\pm$ 311

* $P < 0.05$; ** $P < 0.01$, compared with the WT group (Student's *t* test).

Fig. 2. Blood BaP levels in IE-Cpr-null, liver-Cpr-null, and WT mice, after a single intraperitoneal dose of BaP. Adult male mice were given a single intraperitoneal injection of BaP at 45 mg/kg. Blood samples were collected at various time points after dosing. The IE-Cpr-null (A) and the liver-Cpr-null (B) mice were compared with WT mice in two separate experiments. Values represent means $\pm$ S.D. ($n = 6-8$). *, $P < 0.05$; **, $P < 0.01$, compared with WT mice (Student's t test).

evident in untreated mice, of either strain, in the blot shown in Fig. 3A; however, prolonged exposure of the film indicated the presence of CYP1A1 protein in these untreated mice, as was shown in our previous report (Zhang et al., 2009). BNF treatment led to significant induction of hepatic CYP1A1 protein expression in both mouse strains, at similar levels.

The induction of SI CYP1A1 protein by BNF was accompanied by increases in microsomal BaP hydroxylase activity in WT mice; rates of BaP 3-hydroxylation increased 8-fold, from 11 pmol/min/mg protein in untreated mice to 91 pmol/min/mg protein in BNF-treated mice. A smaller (3-fold) increase in rates was observed for the IE-Cpr-null mice, from 3.3 pmol/min/mg protein in untreated mice to 9.7 pmol/min/mg protein in BNF-treated mice; the rates in the IE-Cpr-null mouse groups were much lower than those seen for the

WT mice, a result reflecting the impact of the loss of CPR protein on microsomal P450 activity in the null strain.

Hepatic microsomal BaP hydroxylase activity did not differ between untreated WT and untreated IE-Cpr-null mice (20–21 pmol/min/mg protein); this activity may be catalyzed by P450s other than CYP1A1, given the barely detectable levels of CYP1A1 in the liver of the untreated mice (Fig. 3A). Hepatic microsomal BaP hydroxylase activity also did not differ between BNF-treated WT mice and BNF-treated IE-Cpr-null mice (284–296 pmol/min/mg protein), consistent with the immunoblot results that indicated similar levels of CYP1A1 protein in these samples (Fig. 3A).

Pharmacokinetic studies were then conducted for oral BaP in BNF-pretreated IE-Cpr-null and WT mice, at the same BNF dose (40 mg/kg by oral gavage) as had been found to cause CYP1A1 induction. For the wild type, BaP was cleared from body much faster in BNF-pretreated mice (Fig. 3B) than in untreated mice (Fig. 1A). Faster BaP clearance was also observed in BNF-pretreated IE-Cpr-null mice (Fig. 3B) than in untreated IE-Cpr-null mice (Fig. 1A). Comparing between BNF-pretreated WT and IE-Cpr-null mice, we found that blood BaP levels were significantly higher in the null strain than in the wild type, at all time points determined (Fig. 3B), and that $AUC_{0-6\text{ h}}$, $C_{\max}$, and $t_{1/2}$ values for blood BaP were 7.0-, 4.3-, and 3.4-fold higher, respectively, in IE-Cpr-null than in WT mice (Table 3). Thus, intestinal P450 plays an important role in the first-pass clearance of oral BaP, both in untreated mice and in mice pre-exposed to a CYP1A1 inducer. Furthermore, a comparison between the untreated and BNF-pretreated mice, for assessment of the magnitude of strain-related differences in AUC (7.0- and 3.1-fold, respectively) and $C_{\max}$ values (4.3- and 1.8-fold, respectively), supports the concept that the contribution of intestinal P450-mediated metabolism to first-pass clearance of oral BaP is even greater in animals pre-exposed to CYP1A1 inducers than it is in untreated animals.

Time Course of CYP1A1 Induction by Orally Administered BaP. The above-mentioned finding of the important role of SI CYP1A1 induction in oral BaP clearance, and BaP itself being a potent CYP1A1 inducer, prompted us to investigate the potential role of BaP-mediated CYP1A1 induction in the first-pass metabolism of oral BaP. We performed immunoblot analyses for CYP1A1 protein expression, to determine whether the onset of CYP1A1 induction by oral BaP was sufficiently early to account for the rapid clearance of BaP, an event that took place within 4 h after dosing, in WT mice (Fig. 1A). As shown in Fig. 4, CYP1A1 expression in the SI was clearly induced (by ~ 3 -fold) as early as 2 h after dosing, a time when CYP1A1 induction was not yet detectable in other tissues (lung, liver, and kidney) examined. By 4 h after dosing, obvious induction was observed in all four

TABLE 2

Pharmacokinetic parameters for intraperitoneally administered BaP in IE-Cpr-null, liver-Cpr-null, and WT mice. Data from Fig. 2 were analyzed for determination of pharmacokinetics parameters. Values represent means $\pm$ S.D. ($n = 6-8$).

Experiment No.	Strain	$T_{\max}$	$C_{\max}$	$t_{1/2}$	$AUC_{0-6\text{ h}}$
		<i>h</i>	<i>ng/ml</i>	<i>h</i>	<i>ng · h/ml</i>
1	WT	0.50	115 $\pm$ 27	3.44 $\pm$ 1.18	309 $\pm$ 64
	IE-Cpr-null	0.56	119 $\pm$ 27	2.91 $\pm$ 1.05	297 $\pm$ 39
2	WT	0.50	128 $\pm$ 36	3.06 $\pm$ 0.52	336 $\pm$ 73
	liver-Cpr-null	0.56	114 $\pm$ 21	2.23 $\pm$ 0.30**	355 $\pm$ 52

** $P < 0.01$, compared with the WT group (Student's t test).

Fig. 3. Effects of CYP1A1 induction on intestinal BaP metabolism and first-pass BaP clearance. A, immunoblot analysis of CYP1A1 expression in mouse liver and small intestine. Liver and SI microsomes were prepared from untreated or BNF-treated adult male WT and IE-Cpr-null mice. Mice in the BNF-treated group were treated with BNF, via daily intraperitoneal injection (once per day at 40 mg/kg each), for three consecutive days. Tissues were obtained at 24 h after the last BNF injection. Microsomal proteins (5 μ g/lane) were analyzed in duplicate. CYP1A1 protein (~59 kDa) was detected using a CYP1A1-specific antibody. B, clearance of orally administered BaP in BNF-pretreated IE-Cpr-null and WT mice. Adult male mice, pretreated with BNF as described in A were given a single oral dose of BaP at 30 mg/kg at 24 h after the last BNF dose. Blood samples were obtained at various time points after BaP dosing for BaP determination. Values represent means $\pm$ S.D. ($n = 6$). **, $P < 0.01$, compared with WT mice (Student's t test).

organs, and by 6 h, the induction had reached the maximum. This result indicated that SI CYP1A1 is substantially induced by oral BaP as early as 2 h after dosing, even at a BaP dose (15 mg/kg) lower than that used for the pharmacokinetics studies (30 mg/kg). Furthermore, the induction in the SI, the portal-of-entry organ, was earlier than in extra-gut organs.

Role of Small-Intestinal P450s in Reducing BaP Tissue Burdens after Oral Exposure. The higher blood BaP levels in IE-Cpr-null mice treated with BaP by the oral route than in identically treated WT mice suggested that corresponding differences would exist in the tissue burden of the toxicant in various target organs. To test this hypothesis, we compared the tissue levels of BaP between IE-Cpr-null and WT mice; BaP was administered orally at 30 mg/kg, and tissues were harvested for analysis of BaP content at either 6 h (Fig. 5A) or 24 h (Fig. 5B) after dosing. At the 6-h time

point, tissue BaP content was significantly higher for the SI (duodenum), liver, and kidney of the IE-Cpr-null mice than for corresponding organs of the WT mice (Fig. 5A). Apparently higher levels were also noted in the colon, lung, and thymus of the null mice, compared with corresponding organs of the WT mice, although statistical significance was not achieved. Likewise, at the 24-h time point, the IE-Cpr-null mice exhibited higher tissue BaP content than did the WT mice, with significant differences in BaP content seen in all organs but thymus (Fig. 5B). The results from the 24-h time point also serve to exclude the possibility of contamination of the SI and colon tissue samples by extracellular BaP from the intestinal tract, given the reported transit time for foods through the gastrointestinal tract: ~15 h in rats (DeSesso and Jacobson, 2001) and ~10 to 11 h in mice (Bellier et al., 2005). Thus, not only can the loss of intestinal P450 activity lead to higher circulating BaP levels but also it can cause significant increases in the tissue burden of the toxicant in the gut and various extra-gut target organs, in mice exposed to BaP through oral ingestion.

Discussion

The metabolism of BaP, and the mechanisms of BaP toxicity, have been the subject of intensive toxicological study. One of the recent breakthroughs in the field, made possible by the development of *Cyp1a1*, *Cyp1a2*, and *Cyp1b1* single- or double-knockout mice, revealed that CYP1A1 plays the major protective role against oral BaP exposure, whereas CYP1B1, located in extrahepatic target tissues such as the bone marrow, is responsible for BaP toxicity (Uno et al., 2004, 2006). In the latter studies, the potential role of small-intestinal CYP1A1 in detoxifying oral BaP was proposed; however, the role of hepatic CYP1A1 in the first-pass metabolism of oral BaP could not be excluded, given the fact the *Cyp1a1* gene deletion occurred in the liver, as well as in the SI, in the *Cyp1a1*-knockout mouse model. Thus, a definitive understanding of the specific roles of the SI in the first-pass metabolism of BaP will entail further studies that use mouse models having tissue-specific suppression of CYP1A1 activity. In that regard, although an SI-specific (or liver-specific) *Cyp1a1*-null mouse model is not yet available, our recently developed IE-Cpr-null mouse (Zhang et al., 2009), in which the activities of all microsomal P450 enzymes, including CYP1A1, are suppressed serves as a valuable alternative. The use of the IE-Cpr-null model, and of the complementary liver-Cpr-null model, has made it possible for us to definitively conclude that P450 enzymes in the SI, but not those in the liver, are critical for protection against systemic exposure to oral BaP.

Our findings also indicate that the induction of CYP1A1 in the SI by oral BaP is a critical event in the protective process. As shown in the present and previous (Uno et al., 2008)

TABLE 3

Pharmacokinetic parameters for orally administered BaP in BNF-pretreated mice

Data from Fig. 3B were analyzed for determination of pharmacokinetics parameters. Values represent means $\pm$ S.D. ($n = 6$).

Strain	T_{max} h	C_{max} ng/ml	$t_{1/2}$ h	AUC _{0-6 h} ng · h/ml
WT	0.92 $\pm$ 0.20	30 $\pm$ 10	0.38 $\pm$ 0.04	54 $\pm$ 11
IE-Cpr-null	1.50 $\pm$ 0.55	129 $\pm$ 43**	1.31 $\pm$ 0.77**	380 $\pm$ 56**

** $P < 0.01$, compared with WT mice (Student's t test).

Fig. 4. Time course of CYP1A1 induction after oral BaP administration. A, adult male WT mice were treated with BaP via oral gavage at a dose of 15 mg/kg. Tissues (liver, lung, kidney, and SI) were obtained at various times after the treatment, and microsomes were prepared for immunoblot analysis of CYP1A1 expression. Microsomal proteins (10 μ g/lane) were analyzed in duplicate. B, densitometry analysis. The average values for the duplicate samples are presented.

studies, constitutive expression of CYP1A1 in the SI and other organs of adult mice is very low. The consequences of the low basal levels of CYP1A1 expression and activity were reflected in our pharmacokinetics studies on oral BaP. At 0.5 or 1 h after oral BaP exposure, there was no significant difference, between IE-Cpr-null and WT mice, in blood BaP levels, although significant differences were found between the two mouse strains at later time points (at 2, 4, and 6 h). These results are in sharp contrast with results from our recent studies on the pharmacokinetics of oral nifedipine, a CYP3A substrate, in the IE-Cpr-null mice and in WT mice (Zhang et al., 2009). For orally administered nifedipine, the largest difference in blood nifedipine levels between IE-Cpr-null and WT mice was found at 0.5 h after dosing.

The pharmacokinetic differences that we have observed between oral BaP and oral nifedipine can be explained by BaP and nifedipine being metabolized in the SI by differing P450s: CYP1A1 and CYP3A, respectively. CYP3A proteins are constitutively expressed at high levels in the mouse and human SI (Zhang et al., 1999, 2003; Paine et al., 2006). Thus, extensive first-pass metabolism mediated by CYP3A enzymes is expected to occur as soon as nifedipine is absorbed in the SI in the WT mice but not in the IE-Cpr-null mice. In contrast, for BaP, a substantial proportion of the ingested compound passes through the SI and liver, without undergoing metabolism, in both WT and IE-Cpr-null mice, until significant induction of CYP1A1 has occurred in the SI.

The positive impact of CYP1A1 induction in the SI on first-pass BaP metabolism was supported by the results of

Fig. 5. BaP levels in various organs from BaP-treated IE-Cpr-null and WT mice. Adult male mice were given a single oral dose of BaP at 30 mg/kg. Tissues were obtained at 6 (A) or 24 h (B) after dosing, for determination of BaP. Values represent means $\pm$ S.D. ($n = 4-6$). *, $P < 0.05$; **, $P < 0.01$, compared with WT mice (Student's t test).

pharmacokinetics studies in WT and IE-Cpr-null mice that had been pretreated with BNF, for induction of CYP1A1 expression. In that experiment, the difference between the two mouse strains, in blood BaP levels, was significant at all examined time points (as early as 0.5 h) after oral BaP administration, in contrast to the delayed appearance of such differences in mice not pretreated with BNF.

Further support for the concept that the induction of SI CYP1A1 protein by oral BaP is important in first-pass BaP clearance was obtained through an examination of the time course of CYP1A1 induction. By 2 h after BaP oral administration, SI CYP1A1 protein levels were already elevated in WT mice; thus, the time course for the induction of CYP1A1 protein expression in the SI is rapid enough to account for the large difference in blood BaP levels between WT and IE-Cpr-null mice at 2 h after BaP dosing, a time when a significant CYP1A1 induction in other organs has not yet occurred.

Our findings also elucidate the role of hepatic P450 enzymes in the first-pass metabolism of BaP, whether administered orally or intraperitoneally. In contrast to the substantial differences in pharmacokinetic parameters seen for oral exposure between WT and IE-Cpr-null mice, there were no significant differences in pharmacokinetic parameters between WT and liver-Cpr-null mice. The lack of a significant involvement of hepatic P450 enzymes in the clearance of oral BaP can be partly explained by the very low basal expression level of CYP1A1 in the liver, as well as the relatively late (compared with induction in the SI) induction of CYP1A1 by oral BaP. In addition, the very high hydrophobicity of BaP may cause the majority of absorbed BaP to be transferred

directly from the SI, through the lymphatic system, into systemic circulation, thus bypassing the first-pass metabolism by hepatic enzymes (Charman et al., 1997). Nevertheless, a comparison of the $C_{\max}$ or AUC values for oral BaP, between IE-Cpr-null mice not pre-exposed to a CYP1A1 inducer (378 ng/ml and 1090 ng · h/ml, respectively), and those pretreated with BNF for CYP1A1 induction (129 ng/ml and 380 ng · h/ml, respectively), strongly suggests that at the level of induced expression, hepatic and other extra-gut organ CYP1A1 also play an important role in the clearance of oral BaP.

Most drugs or toxicants administered via the intraperitoneal route will first enter the mesenteric vein and then be transferred via the portal vein to the liver, where they potentially undergo first-pass metabolism. Thus, the low basal expression of hepatic CYP1A1 may explain, at least in part, the absence of a substantial difference in clearance of intraperitoneal BaP, between liver-Cpr-null and WT mice; the lack of difference was seen both in this study and in a previous study that used the mouse model HRN-null (Arlt et al., 2008), which is similar to the liver-Cpr-null studied here. In addition, no significant difference in clearance of intraperitoneal BaP was seen between our WT and IE-Cpr-null mice, a result supporting the idea that the SI does not play an important role in the metabolism of circulating BaP. However, the low rate of hepatic metabolism of BaP does not suffice to explain why blood levels of BaP achieved after intraperitoneal dosing are generally lower than the blood levels achieved after oral BaP exposure, for any given dose. It is conceivable that, given the high hydrophobicity of BaP, a large proportion of the dosed BaP is trapped in the liver or the abdominal cavity, after intraperitoneal dosing and is then slowly released, through systemic or lymphatic circulation, to metabolic organs for metabolism, or directly excreted; this scenario has been proposed previously in a study of BaP clearance in AhR low-responsive (AhR^d) and high-responsive (AhR^b) mice (Galván et al., 2005).

Our present finding that the suppression of SI metabolism of oral BaP leads to substantial increases in the BaP tissue burden, not only in SI but also in most other tissues examined, provides strong support for the proposed crucial role of SI P450 in protection against systemic exposure to oral BaP. The elevation of BaP tissue levels in the colon is of particular interest. Although the SI is not a common site for tumor formation, the colon, in contrast, is one of the sites with highest tumor incidence in humans. It has long been proposed that high P450 activities in the SI can protect the colon against exposure to orally ingested carcinogens, such as BaP (Kaminsky and Fasco, 1991); the higher BaP content seen in the colon of IE-Cpr-null mice, compared with the WT mice, seems to support this hypothesis. Considering that CPR levels in extra-gut organs were not altered in the IE-Cpr-null mice, the differences between WT and IE-Cpr-null mice in BaP tissue content are probably a result of the differences between the two mouse strains in blood BaP levels. However, it remains to be determined whether BaP metabolism in the SI affects the quantity of BaP remaining in the intestinal lumen, available for absorption into colon epithelium.

In summary, our results indicated that P450 enzymes in the SI, but not those in the liver, play a pivotal role in protection against systemic exposure to oral BaP in mice.

Furthermore, the induction of SI CYP1A1 by BaP is a critical event in the protective process. The absence of SI P450 activity led to increased BaP tissue burdens in mice exposed to orally ingested BaP. Further studies are warranted to determine the impact of this increased tissue BaP burden on the extent of tissue toxicity and the incidence of tumorigenesis in the various extra-gut organs in the IE-Cpr-null model.

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