Cytochrome P450 1B1 Determines Susceptibility to Dibenzo[*a*,*l*]pyrene-Induced Tumor Formation

Jeroen T. M. Buters,*,† Brinda Mahadevan,‡ Leticia Quintanilla-Martinez,§ Frank J. Gonzalez,|| Helmut Greim,† William M. Baird,‡ and Andreas Luch†,⊥

Institut für Toxikologie und Umwelthygiene, Technische Universität München, Lazarettstrasse 62, 80636 München, Germany, GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für Pathologie, Ingolstädter Landstrasse 1, 85764 Oberschleissheim, Germany, Department of Environmental and Molecular Toxicology, 1007 Agriculture and Life Sciences Building, Oregon State University, Corvallis, Oregon 97331-7302, Laboratory of Metabolism, Division of Basic Sciences, National Cancer Institute, Building 37, Room 3E24, Bethesda, Maryland 20892

Received February 21, 2002

Metabolic activation, DNA binding, and tumorigenicity of the carcinogenic polycyclic aromatic hydrocarbon dibenzo[*a*,*l*]pyrene (DB[*a*,*l*]P) catalyzed by murine cytochrome P450 (P450) enzymes were investigated. DNA binding of DB[*a*,*l*]P in human mammary carcinoma MCF-7 and human P450-expressing Chinese hamster V79 cell lines was previously shown to occur preferentially with metabolically generated fjord region DB[*a*,*l*]P-11,12-dihydrodiol 13,14-epoxides (DB[*a*,*l*]PDE). To elucidate different capabilities of murine P450 1A1 and 1B1 for metabolic activation of DB[*a*,*l*]P, V79 cell cultures stably expressing P450s 1A1 or 1B1 from mice were exposed to 10 or 100 nM DB[*a*,*l*]P. Both cell lines transformed DB[*a*,*l*]P to DNA binding intermediates. As with V79 cells expressing the corresponding human P450 enzyme [Luch et al. (1998) *Chem. Res. Toxicol.* **11**, 686−695], murine P450 1B1-catalyzed metabolism and DNA binding proceeded exclusively through generation of fjord region DB[*a*,*l*]PDE. In addition, only DB[*a*,*l*]PDE-derived DNA adducts were found in V79 cells expressing P450 1A1 from mice. This is in contrast to our recent findings with V79 cells expressing P450 1A1 from humans or rats which catalyzed the formation of both highly polar DNA adducts as well as nonpolar DB[*a*,*l*]PDE-DNA adducts. To establish the role of P450 1B1 in DB[*a*,*l*]P-induced tumor formation in vivo, we treated P450 1B1-null and wild-type mice intragastrically and monitored survival rates and appearance of neoplasias in various organs. All wild-type mice ($n = 17$) used in this study developed at least one tumor at one site (tumor rate of 100%). In contrast, 5 of 13 P450 1B1-null mice were observed to be free from any tumor (tumor rate of 62%). The organ sites of tumor formation and the dignity of tumors were different between wild-type and P450 1B1-null mice. Wild-type mice were diagnosed with both benign and malignant tumors of the ovaries, lymphoid tissues, as well as with skin and endometrial hyperplasias, whereas P450 1B1-null mice developed only lung adenomas and endometrial hyperplasias. DNA binding studies using embryonic fibroblasts isolated from these animals provided further evidence that P450 1B1-catalyzed formation of fjord region DB[*a*,*l*]PDE-DNA adducts is the critical step in DB[*a*,*l*]P-mediated carcinogenesis in mice, and probably also in man.

Introduction

Metabolic activation of carcinogenic polycyclic aromatic hydrocarbons (PAHs)[1] is predominantly catalyzed by members of the superfamily of cytochrome P450-dependent monooxygenases (P450) (*1*−*5*). It has been widely demonstrated in the past that all homologous P450 1A1 forms from animals or humans are competent to activate a range of various carcinogenic PAHs through formation of DNA-reactive bay or fjord region diol epoxides (*6*−*10*). Although the P450 1A1 enzyme is virtually not expressed in the absence of inducers (*1*, *2*, *11*, *12*), it may play an important role in long-term induction of tumorigenicity in different organs due to its inducibility by various PAHs (*13*−*15*).

Another enzyme member of the P450 family, P450 1B1, was discovered and detected in various rodent and human tissues in the 90s (*16*−*19*). P450 1B1 displays

* To whom correspondence should be addressed. Current address: Center for Allergy and Environment Munich, Technische Universität München, Biedersteinerstrasse 29, 80802 München, Germany. Phone: +49-89-4140-3487. Fax: +49-89-4140-3453. E-mail: buters@lrz.tum.de.

† Institut für Toxikologie und Umwelthygiene.

‡ Oregon State University.

§ GSF-Forschungszentrum für Umwelt und Gesundheit.

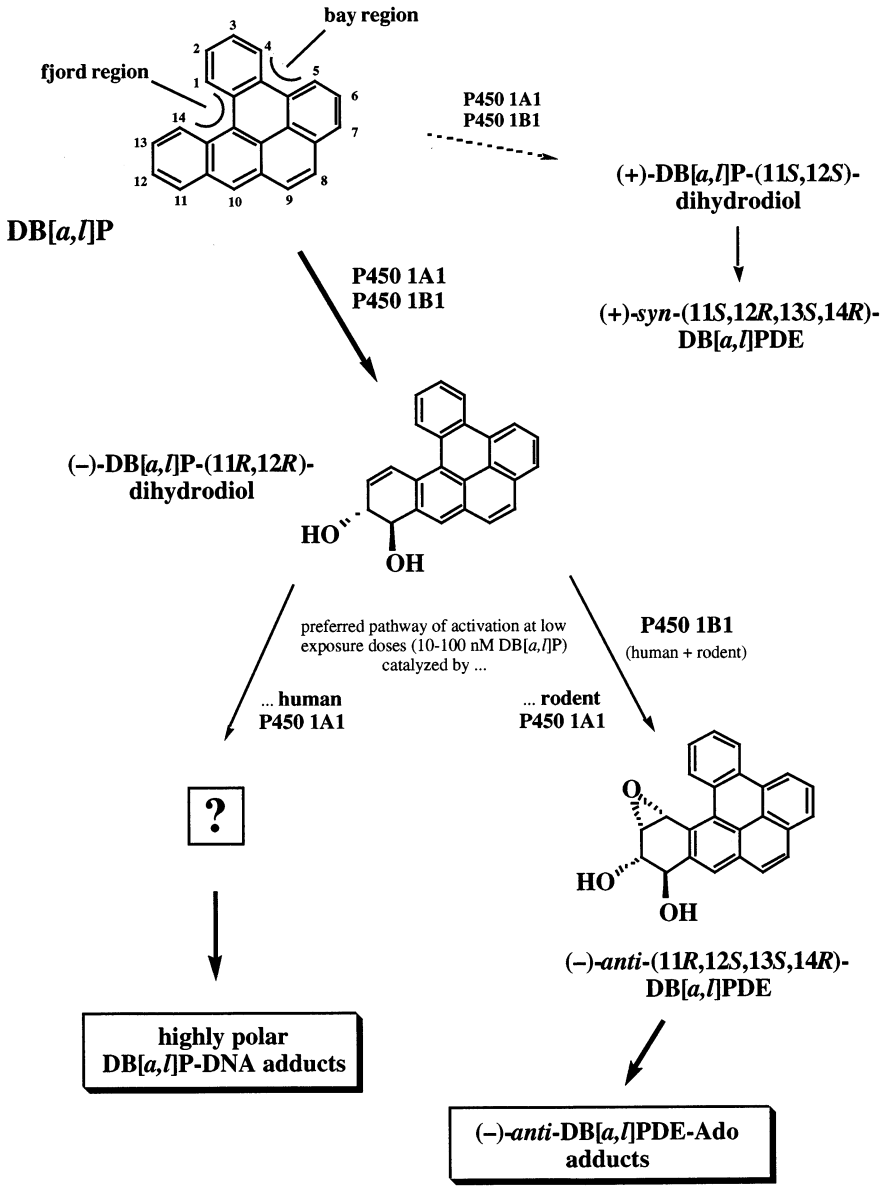
|| National Cancer Institute.

⊥ Present address: Department of Cell Biology, Institute of Chemistry and Cell Biology, Harvard Medical School, 250 Longwood Avenue, SGM 604, Boston, Massachusetts 02115-5731.

[1] Abbreviations: B[*a*]P, benzo[*a*]pyrene; DB[*a*,*l*]P, dibenzo[*a*,*l*]-pyrene; DB[*a*,*l*]PDE, dibenzo[*a*,*l*]pyrene-(11*S*,12*R*)-dihydrodiol (13*S*,-14*R*)-epoxide(s); (+)-*syn*-DB[*a*,*l*]PDE, (+)-*syn*-dibenzo[*a*,*l*]pyrene-(11*S*,-12*R*)-dihydrodiol (13*S*,14*R*)-epoxide; (−)-*anti*-DB[*a*,*l*]PDE, (−)-*anti*-dibenzo[*a*,*l*]pyrene-(11*R*,12*S*)-dihydrodiol (13*S*,14*R*)-epoxide; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMEM, Dulbecco's modified Eagle medium, high glucose-type; HPLC, high-performance liquid chromatography; P450, cytochrome P450; PAH, polycyclic aromatic hydrocarbon.

10.1021/tx020017q CCC: $22.00 © 2002 American Chemical Society
Published on Web 08/08/2002

Scheme 1. Stereoselective Bioactivation of Dibenzo[*a,l*]pyrene in Its Fjord Region, Catalyzed by P450 1A1 and 1B1 Enzymes[a]



[a] The scheme includes activation of DB[*a,l*]P to the diastereomeric fjord region (+)-*syn*- and (−)-*anti*-DB[*a,l*]PDE via the corresponding metabolic precursors, the (+)- and (−)-*trans*-DB[*a,l*]P-11,12-dihydrodiol, respectively. At low exposure doses of the parent PAH (≤100 nM), both enzymes, P450 1A1 and 1B1, predominantly catalyze the formation of the (−)-*trans*-11,12-dihydrodiol enantiomer. This has been previously described in the case of human P450 enzymes (*20*, *21*, *30*) and confirmed in the present study by application of homologous enzymes from mice (see text). The (−)-*trans*-11,12-dihydrodiol subsequently is converted by P450 1B1 exclusively to the (−)-*anti*-DB[*a,l*]PDE to yield predominantly 2′-deoxyadenosine (Ado) adducts. In contrast to human P450 1A1 and similar to rat P450 1A1, exposure of murine P450 1A1 to low doses of DB[*a,l*]P leads mainly to the generation of (−)-*anti*-DB[*a,l*]PDE-derived DNA adducts and not to the formation of as yet unidentified highly polar DNA adducts (cf. Figure 1 and text) (*21*, *30*).

high enzymatic activity toward a wide range of PAHs including the potent carcinogens benzo[*a*]pyrene (B[*a*]P), 7,12-dimethylbenz[*a*]anthracene (DMBA) and dibenzo-[*a,l*]pyrene (DB[*a,l*]P, Scheme 1) (*7*, *20*−*25*). Among these PAHs, the hexacyclic hydrocarbon DB[*a,l*]P has been characterized as the strongest carcinogenic compound tested to date in mouse skin and rat mammary gland model systems (*26*−*29*). Using recombinant human or rat P450-expressing V79 cell lines and applying the ^{33}P-postlabeling assay in conjunction with HPLC separation techniques, we have demonstrated that P450s 1A1 and 1B1 are mainly responsible for the DNA binding of DB-[*a,l*]P through an intermediate formation of the (−)-DB-[*a,l*]P−(11*R*,12*R*)-dihydrodiol (*20*, *21*, *30*). As earlier demonstrated with human mammary carcinoma MCF-7 cells that constitutively express P450 1B1 but not 1A1 (*31*, *32*), at low exposure doses of the parent compound (≤1 μM), human P450 1B1-expressing V79 cells ste-reospecifically catalyze the formation of the (−)-(11*R*,-12*S*,13*S*,14*R*)-DB[*a,l*]P-11,12-dihydrodiol 13,14-epoxide [(−)-*anti*-DB[*a,l*]PDE] via its (−)-*trans*-11,12-dihydrodiol precursor (Scheme 1). In contrast, cell treatment with higher and cytotoxic doses of DB[*a,l*]P was required to detect some small amounts of DNA adducts originating from the diasteromeric (+)−(11*S*,12*R*,13*S*,14*R*)-DB[*a,l*]P-11,12-dihydrodiol 13,14-epoxide [(+)-*syn*-DB[*a,l*]PDE] [Scheme 1 (*30*)]. On the other hand, human P450 1A1-expressing V79 cells catalyze the formation of both fjord

region DB[*a*,*l*]PDE-DNA adducts as well as several highly polar DNA adducts (*20*, *21*, *30*). However, only as yet unidentified highly polar DNA adducts were detected after treatment of these cells with low doses (0.1 μM) of DB[*a*,*l*]P (*20*).

High tumor rates obtained after direct administration of the (−)-DB[*a*,*l*]P−(11*R*,12*R*)-dihydrodiol on mouse skin (*33*) and the finding that exclusively (−)-*anti*-DB[*a*,*l*]PDE-DNA adducts could be detected in mouse skin after application of the parent compound (*34*) provided evidence that tumor initiation by DB[*a*,*l*]P is mainly mediated through metabolic formation of the (−)-*anti*-DB[*a*,*l*]-PDE. Assuming a similar stereo- and regioselectivity of human and murine P450 1A1 and 1B1, respectively, the above-mentioned observations might also lead to the conclusion that P450 1B1 rather than 1A1 mediates the tumorigenicity of DB[*a*,*l*]P. To understand the mechanism of P450-catalyzed activation of this carcinogen in vivo, we have treated P450 1B1-null as well as wild-type mice with low doses of this compound (*35*, *36*). The findings presented in this study provide further evidence and confirm previous observations that P450 1B1-catalyzed formation of (−)-*anti*-DB[*a*,*l*]PDE-DNA adducts is the critical activation step in DB[*a*,*l*]P-induced carcinogenesis.

Experimental Procedures

Caution! *DB[a,l]P is a potent carcinogenic agent and should be handled accordingly.*

Chemicals and Media. DB[*a*,*l*]P (99.8%) was synthesized by Dr. Werner Schmidt, Institut für PAH−Forschung (Greifenberg, Germany). Solvents used for HPLC were of high grade purity for spectroscopy. Nuclease P1 (from *Penicillium citrinum*), human prostatic acid phosphatase (from human semen), apyrase (from *Solanum tuberosum*), proteinase K (from *Tritirachium album*), and snake venom phosphodiesterase I (from *Crotalus atrox*) were purchased from Sigma Chemical Co. RNase T1 (from *Aspergillus oryzae*) and RNase (DNase-free) were obtained from Boehringer Mannheim Co. T4 polynucleotide kinase was obtained from United States Biochemical. [γ-^{33}P]-ATP [2000 Ci (74 TBq)/mmol] was purchased from NEN-Dupont. Dulbecco's modified Eagle medium, high glucose-type (DMEM with 4.5 g of D-glucose/L), antibiotics (penicillin, streptomycin), L-glutamine, and phosphate-buffered saline (PBS without Ca^{2+} or Mg^{2+}; 3.0 mM KCl, 1.5 mM KH_2PO_4, 140 mM NaCl, 8.0 mM Na_2HPO_4, pH 7.0) were obtained from Biochrom-Seromed, and fetal calf serum was purchased from Intergen. Corn oil was obtained from a commercial source (Mazola).

Isolation of Embryonic Fibroblasts from Mice. The generation of P450 1B1-null mice has been previously described (*37*). At day 15 of gestation, pregnant female P450 1B1-null and wild-type mice (mixed genetic background of C57Bl/6 and 129/Sv) were killed by carbon dioxide asphyxiation. The embryos were placed in PBS buffer, and the internal organs and heads were removed. The remaining torsos were minced and placed into 2 mL of 0.25% trypsin (0.02% EDTA) for 30 min at 37°C. The torsos were suspended by pipetting, and the trypsinization was stopped with DMEM, supplemented with 10% fetal calf serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine. The cells were grown at 37°C in 5% CO_2 until confluency and were then trypsinized and stored at −80°C until use.

Cell Culture, Treatment, and Isolation of DNA. Genetically engineered Chinese hamster V79 cells expressing murine P450 1A1 or 1B1 were kindly provided by Prof. Dr. J. Doehmer. Murine P450 1A1-expressing cells were measured with an expression level of 10 ± 2 pmol of P450 1A1/mg of protein (see ref *38* and footnote 2), whereas murine P450 1B1 was instable upon isolation (*39*). These cells, or embryonic fibroblasts from P450 1B1-null or wild-type mice (P450 expression levels too low to be determined), were cultivated in DMEM, supplemented with 1 mM sodium pyruvate, 4 mM L-glutamine, 10% fetal calf serum, 100 units of penicillin/mL, and 100 μg of streptomycin/mL, at 37°C, 7% CO_2, and 90% saturated atmospheric humidity (*20*, *37*). Prior to treatment, V79 cells or embryonic fibroblasts were seeded at a density of 1×10^6 cells/75 cm^2 culture flask and grown overnight in 30 mL of supplemented DMEM. Cells were then treated with 10 or 100 nM DB[*a*,*l*]P by adding this PAH in a total volume of 10 μL of Me_2SO to the medium. After an incubation period of 6 or 24 h, the supernatant was removed and the cells were harvested by trypsinization with 0.025% trypsin and 0.01% EDTA in PBS. The cells were then washed twice with PBS, and the DNA was isolated by applying the RNase/Proteinase K procedure in conjunction with subsequent phenol/chloroform/isoamyl alcohol extraction and purification steps as described earlier (*20*).

^{33}P-Postlabeling of DNA Adducts. The procedure used was as previously described (*20*, *21*, *32*). Briefly, 10 μg of DNA isolated from V79 cells or embryonic fibroblasts after treatment with DB[*a*,*l*]P was digested with nuclease P1 and prostatic acid phosphatase, postlabeled with [γ-^{33}P]ATP (2000 Ci/mmol), cleaved to adducted mononucleotides with snake venom phosphodiesterase I, and prepurified with a Sep-Pak C_{18} catridge (Waters Corp.). Subsequent separation by analytical HPLC (Varian HPLC system equipped with two pumps and an autosampler; Varian Systems, Inc.) was carried out using a C_{18} reversed-phase column (5-μm Ultrasphere ODS, 4.6 × 250 mm). The solvent system consisted of 0.1 M ammonium phosphate buffer (pH 5.5; solvent A) and 10% acetonitrile-90% methanol (solvent B) at a flow rate of 1 mL/min. The gradient for elution of the DNA adducts was as follows: 20−44% solvent B over 20 min; 44−49% solvent B over 40 min; and 49−65% solvent B over 60 min. The radiolabeled nucleotides were detected by an on-line radioisotope flow-detector (Packard Instruments), and the level of DNA binding was calculated based on labeling efficiency of a [^{3}H]B[*a*]P-7,8-dihydrodiol 9,10-epoxide standard.

Carcinogenicity Study. Seven-week-old female wild-type and P450 1B1-null mice (kept in a conventional facility) were treated intragastrically with 1.07 mg/kg DB[*a*,*l*]P (~32 μg/mouse) dissolved in 100 μL corn oil or corn oil alone, once daily for 5 days/week for 3 weeks. The mice were subsequently weighed weekly and observed twice weekly for 12 months for the appearance of tumors. Mice were killed during this period when either a sudden weight loss of >20% or a tumor of >1 cm occurred. After this period, all remaining mice were killed by carbon dioxide asphyxiation. All mice underwent a complete autopsy and were analyzed by histopathology for the presence and identity of tumors. The organs were stored in 10% buffered formalin and embedded in paraffin. Hematoxylin/eosin stained sections were reviewed by a pathologist. The organs analyzed included lungs, thymus, lymph nodes, spleen, liver, pancreas, kidneys, adrenal glands, intestine, uterus and ovaries. Macroscopically detected tumors were embedded separately and examined.

Statistical Analyses. Statistical analyses of the tumor data were conducted using the Fisher's exact test.

Results

DNA Binding of DB[*a*,*l*]P in Murine Cell Cultures. The HPLC elution profiles of the ^{33}P-postlabeled DNA adducts formed in fibroblasts from P450 1B1-null and wild-type mice or in V79 cells stably expressing murine P450 1A1 or 1B1 are shown in Figure 1. Embryonic fibroblasts isolated from P450 1B1-null mice failed

[2] Krebsfänger, N., Buters, J. T. M., and Doehmer, J. Quantitation of cytochrome P450 contents of genetically engineered V79 cell lines by CO difference spectra. *Biochem. Pharmacol.*, in review.

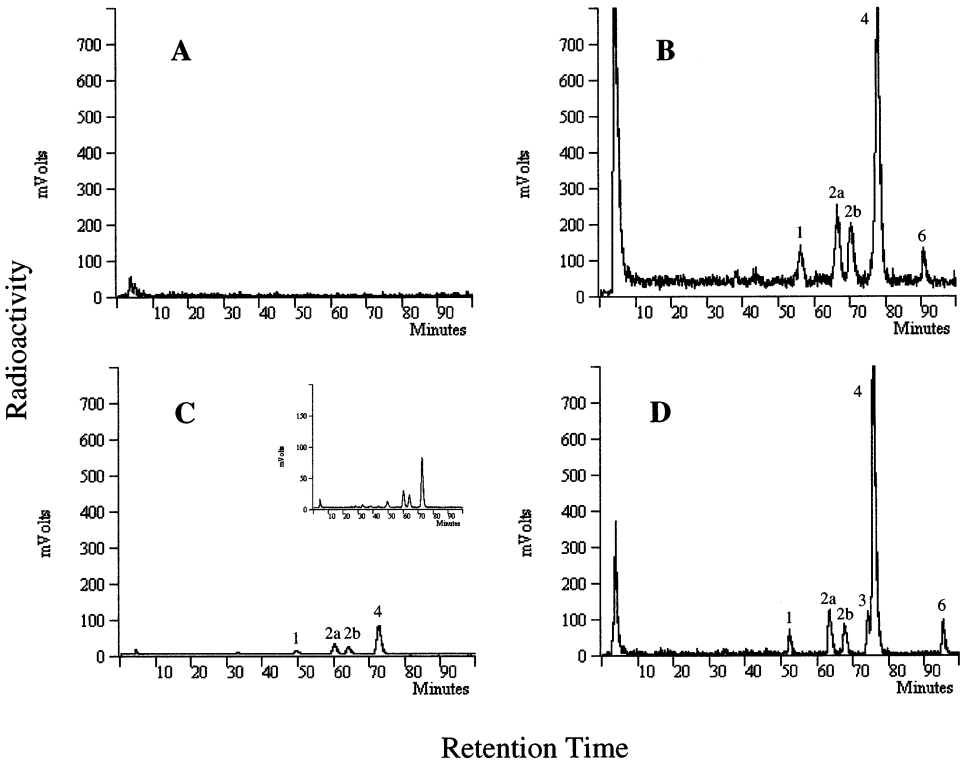


Figure 1. Representative HPLC elution profiles of ^{33}P-postlabeled DNA adducts of DB[*a*,*l*]P formed in embryonic fibroblasts of mice or in Chinese hamster V79 cells stably expressing murine P450 1A1 or 1B1: (A) DNA adducts formed in embryonic fibroblasts from P450 1B1-null mice 24 h after treatment with 100 nM DB[*a*,*l*]P; (B) DNA adducts formed in embryonic fibroblasts from wild-type mice 24 h after treatment with 100 nM DB[*a*,*l*]P; (C) DNA adducts formed in murine P450 1A1-expressing V79 cells 24 h after treatment with 100 nM DB[*a*,*l*]P; (D) DNA adducts formed murine P450 1B1-expressing V79 cells 24 h after treatment with 100 nM DB[*a*,*l*]P. DB[*a*,*l*]PDE-DNA adducts are labeled with arabic numerals (as described in Results). Inset in panel C depicts adduct profile with increased resolution. HPLC conditions are described in Experimental Procedures.

Table 1. Total DNA Binding in Embryonic Murine Fibroblasts or V79 Cells Stably Expressing Murine P450s 6 and 24 h after Exposure to DB[*a*,*l*]P[a]

	10 nM DB[*a*,*l*]P		100 nM DB[*a*,*l*]P	
type of cells treated	6 h	24 h	6 h	24 h
wild-type fibroblasts	1.7	17	4.4	30
P450 1B1 knockout fibroblasts	n.d.	n.d.	n.d.	n.d.
murine P450 1A1-expressing V79 cells	2.9	5.0	4.8	6.1
murine P450 1B1-expressing V79 cells	11	19	8.8	37

[a] All data presented are reported in picomoles of adducts per milligrams of DNA and represent the mean out of two independent sets of treatments with at least two postlabeling reactions per set; n.d., no DNA adducts detected.

to catalyze any DNA binding of DB[*a*,*l*]P (Figure 1A). As listed in Table 1 and depicted by the profile in Figure 1A, no stable DB[*a*,*l*]P-DNA adducts could be detected at any treatment dose or incubation time applied in this study. In contrast, embryonic fibroblasts isolated from wild-type mice were competent for metabolic transformation of DB[*a*,*l*]P toward DNA-reactive intermediates. As shown in Figure 1B and listed in Table 1, the DNA binding catalyzed by wild-type fibroblasts was high and revealed a concentration- and time-dependent increase in the total amounts of adducts formed upon exposure to DB[*a*,*l*]P.

The DNA adduct profile obtained in murine wild-type fibroblasts (Figure 1B) is identical to the profile that was found after treatment of murine P450 1B1-expressing V79 cells (Figure 1D). Although a little shift between the peaks of both profiles can be observed, the identity of both sets of DNA adducts was confirmed by co-chromatography of aliquots of both samples (data not shown). Only late eluting DB[*a*,*l*]PDE-DNA adducts possessing retention times of about 50−100 min were detected. As observed and characterized previously with human P450 1B1-expressing V79 cells (*20*, *21*) or human mammary carcinoma MCF-7 cells (*32*), all adducts in this elution range originate from intermediate formation of fjord region DB[*a*,*l*]PDE. The four peaks labeled with 1, 2a, 2b, and 4 are formed from (−)-*anti*-DB[*a*,*l*]PDE, whereas the two additional peaks labeled with 3 and 6 are due to covalent interaction of (+)-*syn*-DB[*a*,*l*]PDE with DNA (the peak numbering system out of refs *20* and *32* is used to allow direct comparison with results presented in our earlier studies). At all doses and time points investigated in this study, no DNA adducts eluted with a retention time shorter than 50 min.

Irrespective of the concentration of DB[*a*,*l*]P used for cell treatment in this study (10 or 100 nM) or the time of exposure (6 or 24 h), the elution profiles obtained from murine P450 1A1-expressing V79 cells only contained DB[*a*,*l*]PDE-derived DNA adducts (Figure 1C). In particular, no polar DB[*a*,*l*]P-DNA adducts were found in this cell line that would have passed the HPLC column within the first 40 min under the polar elution gradient conditions used. Furthermore, quantification of P450 1A1-catalyzed DNA binding (Table 1) revealed only a low concentration- and time-dependent increase of the total amounts of adducts formed. Comparison of the DNA binding found in the murine P450 1B1 expressing V79 cell line confirmed the findings obtained earlier with human P450-expressing cells. Although we were not successful in determining the murine P450 1B1 expres-

Table 2. Numbers of Female Wild-Type and P450 1B1−Null Mice Found with Tumors after Treatment with 1.07 mg/kg of DB[*a*,*l*]P[a]

pathology	wild-type mice with tumors (%)[b]	P450 1B1-null mice with tumors (%)[b]
ovary	12 (71)	0 (0)
granulosa cell tumor	10	0
kystoma	1	0
tubulostromal adenoma	1	0
hemangiosarcoma	1	0
lymphoma	5 (29)	1 (8)
lymphoblastic	3	0
follicular	2	1
liver	1 (6)	1 (8)
adenoma	1	0
hemangioma	0	1
skin	8 (47)	0 (0)
hyperplasia, verruciform		
uterus	5 (29)	5 (38)
hemangiosarcoma	2	0
endometrial cystic hyperplasia	3	5
lung	0 (0)	5 (38)
adenoma		
mice with any tumor	17 (100)	8 (62)

[a] Mice could have developed multiple tumor sites and several distinctive tumors of the same entity in one organ. Tumors of the same entity in one organ were counted once. Mice could have died during the observation time (up to 12 months) or were euthanised at the end of the experiment (survival curve is shown in Figure 2). Statistical analyses performed according to the Fisher's exact test gave the following results: highly significant differences in the two groups of animals for tumors of the ovary ($p = 0.000\ 07$), skin ($p = 0.004$) and lung ($p = 0.009$); no statistically significant differences for lymphoma ($p = 0.15$), tumors of liver ($p = 0.8$) and uterus ($p = 0.8$), respectively. [b] Percent of animals out of group of wild-type mice ($n = 17$) or P450 1B1 knockout mice ($n = 13$) that developed the particular tumor entity are given in parentheses.

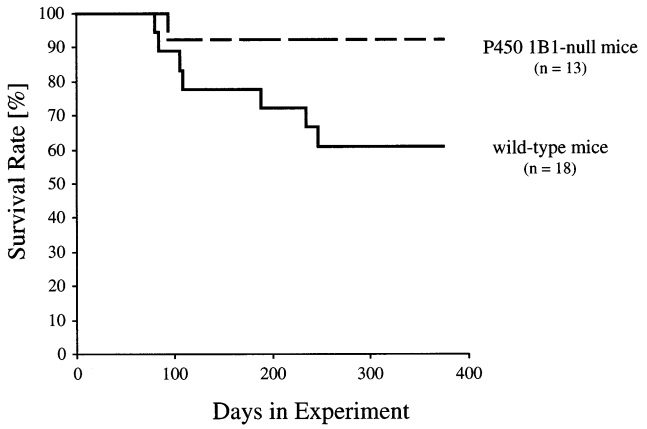


Figure 2. Survival rate (%) of female wild-type or P450 1B1-null mice during a time period of 12 months after intragastrically application of 1.07 mg/kg DB[*a*,*l*]P, once daily for 5 days/week for 3 weeks.

sion level in V79 cells due to the known instability of this enzyme upon isolation processes (*39*), all other P450 enzyme-expressing cell lines constructed by employment of the same method were measured to express always a similar level of about 10 ± 2 pmol of P450/mg of protein (*38*). Assuming murine P450 1B1 not to be an exception in this regard, this enzyme catalyzes a considerably higher DNA binding of DB[*a*,*l*]P compared to P450 1A1 at exposure doses as low as 10 and 100 nM PAHs (*20*).

Tumor Formation in Mice Induced by DB[*a*,*l*]P. Tumor formation sites (tumor entities) and tumor rates found in mice 12 months after starting a 3-week treatment protocol with 1.07 mg/kg/day DB[*a*,*l*]P are compiled in Table 2. As shown in the survival curve (Figure 2), only a single P450 1B1-null mouse (out of 13) died prematurely 3 months after the treatment began (survival rate of 92%). This single mouse did not show any pathologic abnormalities at all. In contrast, during the observation period, seven individuals died in the group of 18 wild-type mice (survival rate of 61%). From these animals, all P450 1B1-null mice but only 17 wild-type mice were subjected to histopathologic evaluation. As confirmed by pathological analysis, all wild-type mice ($n = 17$) developed at least one tumor at one site (tumor rate of 100%), whereas 62% of the P450 1B1-null mice were found with at least one neoplasia at one organ site (Table 2). On the other hand, solvent control groups of mice of both genotypes treated with 100 μL corn oil only hardly developed any tumor-like lesion. Only four hyperplasias were found in 27 individuals. In detail, four wild-type mice were found with one of the following tumor entities each: lung adenoma, liver adenoma, follicular lymphoma, and endometrial cystic hyperplasia, respectively (data not shown).

Comparison of the organ sites of DB[*a*,*l*]P-induced tumor formation and the individual tumor entities revealed considerable differences between wild-type and P450 1B1-null mice (Table 2). Whereas P450 1B1-null mice developed mainly benign tumors of uterus (endometrial cystic hyperplasia) and lung (adenoma), wild-type mice were diagnosed with malignant lymphomas and both benign and malignant tumors of the ovary and uterus as well as with some benign tumors of the liver (adenoma) and skin (papilloma), respectively (Table 2).

The ovary tumors observed in wild-type mice originated mainly from stromal cells (granulosa cell tumors), only two lesions arose from epithelia (tubular adenoma, kystoma) or endothelial cells (hemangiosarcoma). None of these neoplasms could be detected in any of the P450 1B1-null mice exposed to DB[*a*,*l*]P. As with ovary tumors, hyperplasias of the skin (papillomas) and malignant hemangiosarcomas of the uterus were found in wild-type mice only (Table 2). In addition, 29% of the wild-type mice developed lymphomas, whereas only a single P450 1B1-null mouse was diagnosed with this kind of neoplasia. The lymphomas observed in wild-type mice were char-

acterized in part as lymphoblastic (mostly T cell origin) or follicular (large B-cell lymphomas). In contrast to the lesions mentioned above, cystic hyperplasias of the endometrium have been observed irrespective of the P450 1B1 expression status of the rodents, and lung adenomas were exclusively found in P450 1B1-null mice in response to the exposure to DB[*a*,*l*]P (Table 2).

Discussion

About 20 years after Masuda and Kagawa noticed for the first time that the fjord region hexacyclic hydrocarbon DB[*a*,*l*]P is a stronger mouse carcinogen than the well-known bay region hydrocarbon B[*a*]P (*40*), its extraordinarily high potency was confirmed by comparison to a range of different carcinogenic PAHs in various rodent tumor models (*26*−*29*, *41*). On the basis of these animal-related observations and due to its ubiquitous occurrence in the human environment (*42*−*49*) there is a concern that long-term exposure to DB[*a*,*l*]P may pose a risk to human health.

Ample evidence exists that carcinogenic PAHs such as DB[*a*,*l*]P not only induce tumor formation in rodents but also in man (*50*). Studies on the mode of action of these compounds revealed that, in general, tumor initiation occurs via metabolic activation to electrophilically reactive intermediates such as vicinal dihydrodiol epoxides that subsequently damage DNA by forming stable or depurinating DNA adducts (*3*, *5*, *51*, *52*). For instance, the strong carcinogenicity of DB[*a*,*l*]P observed in epithelial organs or lungs of rodents (*26*−*29*, *41*) correlates with the level of DB[*a*,*l*]P-DNA adducts formed in these tissues (*41*, *53*−*56*). The adducts detected originated almost exclusively from fjord region 11,12-dihydrodiol 13,14-epoxides of DB[*a*,*l*]P, which have also been identified as extremely active direct-acting mutagens in vitro (*57*, *58*) and strong carcinogens in vivo (*59*−*61*).

The oxidation steps required for metabolic activation of carcinogenic PAHs toward dihydrodiol epoxides are predominantly catalyzed by P450 enzymes, especially P450 1A1 and 1B1 (Scheme 1) (*22*, *24*, *25*, *62*−*64*). Since these two major activating P450 enzymes are expressed in many mammalian tissues either upon aryl hydrocarbon receptor-mediated induction (P450 1A1) (*65*, *66*) or both constitutively and after receptor-mediated induction (P450 1B1) (*18*, *19*, *65*, *67*, *68*), metabolism and DNA binding studies applying heterologous P450 1A1 or 1B1 expression systems may provide indirect evidence for activation routes of carcinogenic PAHs possibly occurring also in the respective tissues in vivo. In addition, these studies clearly would uncover any species differences in P450-mediated regio- and stereoselectivity in PAH derivatization. Rat and human P450-expressing Chinese hamster V79 cell lines were previously used to elucidate the role of individual P450 enzymes in promoting DB[*a*,*l*]P-dependent cyto- and genotoxicity (*20*, *21*, *69*, *70*). Whereas human P450 1B1 metabolically activated DB[*a*,*l*]P almost exclusively on the fjord region to produce stereoselectively high amounts of (−)-*anti*-DB[*a*,*l*]PDE and low amounts of (+)-*syn*-DB[*a*,*l*]PDE (Scheme 1), both human and rat P450 1A1 formed adducts of both diastereomeric DB[*a*,*l*]PDE as well as several highly polar DNA adducts as yet unidentified (*20*, *21*). Similar to (−)-*anti*-DB[*a*,*l*]PDE-DNA adducts, these highly polar adducts have been found to originate from intermediate formation of the (−)-DB[*a*,*l*]P−(11*R*,12*R*)-dihydrodiol (Scheme 1) (*30*). Consistently, studies on the metabolic activation of both *trans*-11,12-dihydrodiol enantiomers catalyzed by P450 1A1-containing liver preparations from rats (*71*, *72*), human mammary carcinoma MCF-7 cells that constitutively express P450 1B1 but not 1A1 (*20*, *73*), or P450 1A1- and 1B1-expressing V79 cell lines (*20*, *21*) revealed that DNA binding and mutagenic activity were mediated almost exclusively by the (−)-*trans*-11,-12-dihydrodiol. In addition, studies on tumor formation in mouse skin confirmed these enantiospecific differences seen in the genotoxicity assays: the (−)-*trans*-11,12-dihydrodiol was clearly identified as the biologically active species among both enantiomers in the mouse skin tumor initiation-promotion assay (*33*).

In the present study, we treated murine P450 1A1 or 1B1-expressing V79 cells and embryonic fibroblasts from wild-type or P450 1B1-null mice with low concentrations of DB[*a*,*l*]P. As previously determined using the Neutral Red assay (*70*), DB[*a*,*l*]P displays a strong cytotoxic activity in human P450 1A1- and 1B1-expressing V79 cells with EC_{50} values of about 18 and 48 nM, respectively. By monitoring the murine P450-expressing cell lines used in this study, the same assay again confirmed the strong effect of DB[*a*,*l*]P (EC_{50} values of about 15 and 16 nM for murine P450 1A1- and 1B1-expressing cells, respectively; data not shown). Similar to cytotoxicity, the individual DNA adduct profile found after treatment of these two mouse cell lines (Figure 1) resembles the findings previously achieved in experiments exposing human P450 1A1- and 1B1-expressing V79 cell lines to DB[*a*,*l*]P. As with human P450 1B1, the homologous enzyme cloned from mice exclusively catalyed the formation of DB[*a*,*l*]PDE-derived DNA adducts (Scheme 1, Figure 1D). However, no highly polar DNA adducts could be detected in murine P450 1A1-expressing cells after exposure to 10 or 100 nM DB[*a*,*l*]P (Figure 1C). Only DB-[*a*,*l*]PDE-derived adducts were formed. This is in contrast to human P450 1A1-expressing cells that form predominantly highly polar DB[*a*,*l*]P-DNA adducts after exposure to 100 nM DB[*a*,*l*]P (*20*). However, treatment of V79 cells expressing P450 1A1 from rats with doses of DB[*a*,*l*]P as low as 10−100 nM resulted in the formation of DB-[*a*,*l*]PDE-DNA adducts in high excess (*20*). At low exposure doses, the regioselectivity in metabolic conversion of DB[*a*,*l*]P proceeds therefore with similar regioselectivity for both rodent P450 1A1 enzymes leading mainly to DB[*a*,*l*]PDE-DNA adducts, whereas human P450 1A1 exhibits an alternative route of activation leading to polar DB[*a*,*l*]P-DNA adducts (Scheme 1).

As mentioned above, DB[*a*,*l*]P is a very strong carcinogen in mouse skin and rat mammary gland. Intramammillary injection of this PAH in rats and measurement of the DNA adducts formed in several organs 2 days later revealed that only fjord region DB[*a*,*l*]PDE-derived DNA adducts were detectable in extrahepatic tissues such as lung, heart, pancreas, and bladder (*53*, *54*). On the basis of our previous and the present DNA binding studies in V79 cell lines, formation of only DB[*a*,*l*]PDE-DNA adducts in extrahepatic tissues is consistent with the constitutive expression of P450 1B1 in these organs. This enzyme was first discovered in mouse (*16*, *17*, *68*) and subsequently identified in rat (*19*, *74*) and human tissues (*18*, *75*). It can be detected in steroidogenic tissues (e.g., adrenal, ovary, testes), steroid-responsive tissues (e.g., uterus, breast, prostate), and other extrahepatic mammalian tissues such as bone marrow, lung, heart, kidney,

spleen, thymus, and skin (*18*, *19*, *22*, *76*−*78*). To unravel its role in DB[*a*,*l*]P-induced carcinogenesis in mice, we have treated P450 1B1-null mice (*37*) with low doses and subsequently monitored the tumor formation in various organs (Table 2). P450 1B1-null mice were protected against DB[*a*,*l*]P-induced tumors in most tissues. In particular, both benign and malignant tumors of the ovary, the skin and lymphoid tissues were almost solely found in their wild-type counterparts. Striking was the high rate of granulosa cell ovarian tumors in wild-type mice. These tumors originate from stromal fibroblasts that are known to express only P450 1B1 and no P450 1A1, not even after treatment with aryl hydrocarbon receptor-binding compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (*67*, *79*, *80*). Isolation of embryonic fibroblasts from wild-type or P450 1B1-null mice and exposure to DB[*a*,*l*]P confirmed that neither P450 1A1 nor 1B1 was present in P450 1B1-null fibroblasts. No DNA adducts were found that could have been derived from metabolic activation of DB[*a*,*l*]P catalyzed by either of these two different P450 enzymes (Figure 1A). In contrast, wild-type fibroblasts contained several DB[*a*,*l*]-PDE-DNA adducts (Figure 1B). This is due to the catalytic activity of P450 1B1 that is both constitutively expressed and aryl hydrocarbon receptor-induced, whereas P450 1A1 is not expressed in these cells at all (*67*). On the basis of its tissue-specific expression (cf. above) and the striking differences seen in tumor formation in wild-type and knockout mice, P450 1B1-mediated metabolic activation of DB[*a*,*l*]P toward the DNA-damaging, mutagenic and directly carcinogenic (−)-*anti*-DB[*a*,*l*]PDE intermediate (Scheme 1) is thus likely to play a pivotal role in tumor induction in ovary, skin, and central (bone marrow) or peripheral lympoidogenic tissues.

Despite the fact that P450 1B1-null mice developed less DB[*a*,*l*]P-induced tumors than wild-type mice, they were diagnosed with more neoplasias compared to the control group. In particular, benign tumors of the endometrium (cystic hyperplasia) and lung (adenoma) were found in 38% of these animals (Table 2). Benign tumors of the uterus were seen with similar frequencies in wild-type mice indicating the absence of a clear relationship to the P450 1B1 status of the tumor bearing mice. Cell proliferation in steroid-responsive tissues such as endometrium especially may rely on different mechanisms, i.e., epigenetically induced growth stimulation by endogenous estrogens or DNA damage induced through tissue-specific formation of catecholestrogens and estrogen quinones (*81*−*83*). However, it is important to note that control animals treated with corn oil only developed hardly any tumor-like lesion, even so in steroid-responsive tissues such as endometrium (cf. Results). On the other hand, DB[*a*,*l*]P-dependent tumor formation in lung was only observed in P450 1B1-null mice and thus might be due to PAH-mediated induction of P450 1A1 in this organ (*37*), and subsequent activation toward DB[*a*,*l*]PDE-DNA adducts (Figure 1C). Further studies will be undertaken in order to clarify the role of P450 1A1 during DB[*a*,*l*]P-induced tumor formation in the lungs and endometrium of P450 1B1-null mice.

In summary, the present study has revealed a clear relationship between the presence of P450 1B1 and DB-[*a*,*l*]P-dependent tumorigenicity in ovaries, lymphoid tissues and skin from mice after intragastrical administration of low doses of this PAH. Exactly the same organs were previously found to be also susceptible to P450 1B1-mediated tumorigenicity of DMBA (*37*). Although the numbers of animals included in the present study were small, no tumors were found in ovaries and skin and only one B-cell lymphoma of a mesenteric lymph node was diagnosed in P450 1B1-null mice in response to exposure to DB[*a*,*l*]P. In contrast, 71, 47, and 29% of wild-type mice developed tumors in these organs. As demonstrated with human P450 1B1-expressing cell cultures (*20*, *21*) and confirmed in the present study by application of murine P450 1B1-expressing cell cultures and cultures from embryonic fibroblasts isolated from wild-type mice, DB-[*a*,*l*]P is stereoselectively converted to produce high excesses of (−)-*anti*-DB[*a*,*l*]PDE-DNA adducts. These data therefore suggest that P450 1B1-dependent formation of the DNA-reactive (−)-*anti*-DB[*a*,*l*]PDE represents the critical step in DB[*a*,*l*]P-induced tumor formation in ovaries, skin, and lymphoid tissues in mice.

Acknowledgment. This study was supported by the Deutsche Forschungsgemeinschaft (DFG) Grants DO 242/9-1 and 242/9-3 (J.T.B.), and by the National Cancer Institute (NCI) Grants CA40228 and CA28825 (W.M.B.).

References

(1) Guengerich, F. P., and Shimada, T. (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.* **4**, 391−407.

(2) Guengerich, F. P. (2000) Metabolism of chemical carcinogens. *Carcinogenesis* **21**, 345−351.

(3) Gonzalez, F. J., and Gelboin, H. V. (1994) Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metab. Rev.* **26**, 165−183.

(4) Thakker, D. R., Yagi, H., Levin, W., Wood, A. W., Conney, A. H., and Jerina, D. M. (1985) Polycyclic aromatic hydrocarbons: metabolic activation to ultimate carcinogens. In *Bioactivation of Foreign Compounds* (Anders, M. W., Ed.) pp 177−242, Academic Press, New York.

(5) Thakker, D. R., Levin, W., Yagi, H., Wood, A. W., Conney, A. H., and Jerina, D. M. (1988) Stereoselective biotransformation of polycyclic aromatic hydrocarbons to ultimate carcinogens. In *Stereochemical Aspects of Pharmacologically Active Compounds* (Wainer, A. W., and Dryer, D., Eds.) pp 271−296, Marcel Dekker, New York.

(6) Shimada, T., Martin, M. V., Pruess-Schwartz, D., Marnett, L., and Guengerich, F. P. (1989) Roles of individual human cytochrome P-450 enzymes in the bioactivation of benzo[*a*]pyrene, 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene, and other dihydrodiol derivatives of polycyclic aromatic hydrocarbons. *Cancer Res.* **49**, 6304−6312.

(7) Seidel, A., Soballa, V. J., Raab, G., Frank, H., Greim, H., Grimmer, G., Jacob, J., and Doehmer, J. (1998) Regio- and stereoselectivity in the metabolism of benzo[*c*]phenanthrene mediated by genetically engineered V79 Chinese hamster cells expressing rat and human cytochromes P450. *Environ. Toxicol. Pharmacol.* **5**, 179−196.

(8) Shou, M., Krausz, K. W., Gonzalez, F. J., and Gelboin, H. V. (1996) Metabolic activation of the potent carcinogen dibenzo[*a*,*h*]anthracene by cDNA-expressed human cytochromes P450. *Arch. Biochem. Biophys.* **328**, 201−207.

(9) Shou, M., Krausz, K. W., Gonzalez, F. J., and Gelboin, H. V. (1996) Metabolic activation of the potent carcinogen dibenzo[*a*,*l*]pyrene by human recombinant cytochromes P450, lung and liver microsomes. *Carcinogenesis* **17**, 2429−2433.

(10) Shou, M. G., Korzekwa, K. R., Crespi, C. L., Gonzalez, F. J., and Gelboin, H. V. (1994) The role of 12 cDNA-expressed human, rodent, and rabbit cytochromes P450 in the metabolism of benzo-[*a*]pyrene and benzo[*a*]pyrene *trans*-7,8-dihydrodiol. *Mol. Carcinog.* **10**, 159−168.

(11) Larsen, M. C., Angus, W. G. R., Brake, P. B., Eltom, S. E., Sukow, K. A., and Jefcoate, C. R. (1998) Characterization of CYP1B1 and CYP1A1 expression in human mammary epithelial cells: role of the aryl hydrocarbon receptor in polycyclic hydrocarbon metabolism. *Cancer Res.* **58**, 2366−2374.

(12) Gonzalez, F. J. (1993) Cytochromes P450 in humans. In *Cytochrome P450* (Schenkman, J. B., and Greim, H., Eds.) pp 239−257, Springer-Verlag, Berlin.

(13) Ioannides, C., and Parke, D. V. (1993) Induction of cytochrome P4501 as an indicator of potential chemical carcinogenesis. *Drug Metab. Rev.* **25**, 485−501.

(14) Einolf, H. J., Story, W. T., Marcus, C. B., Larsen, M. C., Jefcoate, C. R., Greenlee, W. F., Yagi, H., Jerina, D. M., Amin, S., Park, S. S., Gelboin, H. V., and Baird, W. M. (1997) Role of cytochrome P450 enzyme induction in the metabolic activation of benzo[*c*]-phenanthrene in human cell lines and mouse epidermis. *Chem. Res. Toxicol.* **10**, 609−617.

(15) Marston, C. P., Pereira, C., Ferguson, J., Fischer, K., Hedstrom, O., Dashwood, W.-M., and Baird, W. M. (2001) Effect of a complex environmental mixture from coal tar containing polycyclic aromatic hydrocarbons (PAH) on the tumor initiation, PAH-DNA binding and metabolic activation of carcinogenic PAH in mouse epidermis. *Carcinogenesis* **22**, 1077−1086.

(16) Pottenger, L. H., and Jefcoate, C. R. (1990) Characterization of a novel cytochrome P450 from the transformable cell line, C3H/10T1/2. *Carcinogenesis* **11**, 321−327.

(17) Savas, U., Bhattacharyya, K. K., Christou, M., Alexander, D. L., and Jefcoate, C. R. (1994) Mouse cytochrome P450EF, representative of a new 1B subfamily of cytochrome P-450s: cloning, sequence determination, and tissue expression. *J. Biol. Chem.* **269**, 14905−14911.

(18) Sutter, T. R., Tang, Y. M., Hayes, C. L., Wo, Y.-Y. P., Jabs, E. W., Li, X., Yin, H., Cody, C. W., and Greenlee, W. F. (1994) Complete cDNA sequence of a human dioxin-inducible mRNA identifies a new gene subfamily of cytochrome P450 that maps to chromosome 2. *J. Biol. Chem.* **269**, 13092−13099.

(19) Bhattacharyya, K. K., Brake, P. B., Eltom, S. E., Otto, S. A., and Jefcoate, C. R. (1995) Identification of a rat adrenal cytochrome P450 active in polycyclic aromatic hydrocarbon metabolism as rat CYP1B1: demonstration of a unique tissue specific pattern of hormonal and aryl hydrocarbon receptor-linked regulation. *J. Biol. Chem.* **270**, 11595−11602.

(20) Luch, A., Coffing, S. L., Tang, Y. M., Schneider, A., Soballa, V., Greim, H., Jefcoate, C. R., Seidel, A., Greenlee, W. F., Baird, W. M., and Doehmer, J. (1998) Stable expression of human cytochrome P450 1B1 in V79 Chinese hamster cells and metabolically catalyzed DNA adduct formation of dibenzo[*a*,*l*]pyrene. *Chem. Res. Toxicol.* **11**, 686−695.

(21) Luch, A., Kishiyama, S., Seidel, A., Doehmer, J., Greim, H., and Baird, W. M. (1999) The K-region *trans*-8,9-diol does not significantly contribute as an intermediate in the metabolic activation of dibenzo[*a*,*l*]pyrene to DNA-binding metabolites by human cytochrome P450 1A1 or 1B1. *Cancer Res.* **59**, 4603−4609.

(22) Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., Hecht, S. S., and Guengerich, F. P. (1996) Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* **56**, 2979−2984.

(23) Shimada, T., Watanabe, J., Kawajiri, K., Sutter, T. R., Guengerich, F. P., Gillam, E. M., and Inoue, K. (1999) Catalytic properties of polymorphic human cytochrome P450 1B1 variants. *Carcinogenesis* **20**, 1607−1613.

(24) Shimada, T., Gillam, E. M., Oda, Y., Tsumura, F., Sutter, T. R., Guengerich, F. P., and Inoue, K. (1999) Metabolism of benzo[*a*]-pyrene to *trans*-7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene by recombinant human cytochrome P450 1B1 and purified liver epoxide hydrolase. *Chem. Res. Toxicol.* **12**, 623−629.

(25) Shimada, T., Oda, Y., Gillam, E. M., Guengerich, F. P., 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.

(26) Cavalieri, E. L., Rogan, E. G., Higginbotham, S., Cremonesi, P., and Salmasi, S. (1989) Tumor-initiating activity in mouse skin and carcinogenicity in rat mammary gland of dibenzo[*a*]pyrenes: the very potent environmental carcinogen dibenzo[*a*,*l*]pyrene. *J. Cancer Res. Clin. Oncol.* **115**, 67−72.

(27) Cavalieri, E. L., Higginbotham, S., RamaKrishna, N. V. S., Devanesan, P. D., Todorovic, R., Rogan, E. G., and Salmasi, S. (1991) Comparative dose−response tumorigenicity studies of dibenzo[*a*,*l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene, benzo-[*a*]pyrene and two dibenzo[*a*,*l*]pyrene dihydrodiols in mouse skin and rat mammary gland. *Carcinogenesis* **12**, 1939−1944.

(28) Higginbotham, S., RamaKrishna, N. V. S., Johansson, S. L., Rogan, E. G., and Cavalieri, E. L. (1993) Tumor-initiating activity and carcinogenicity of dibenzo[*a*,*l*]pyrene versus 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene at low doses in mouse skin. *Carcinogenesis* **14**, 875−878.

(29) LaVoie, E. J., He, Z.-M., Meegalla, R. L., and Weyand, E. H. (1993) Exceptional tumor-initiating activity of 4-fluorobenzo[*j*]fluoranthene on mouse skin: comparison with benzo[*j*]fluoranthene, 10-fluorobenzo[*j*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*l*]pyrene and 7,12-dimethylbenz[*a*]anthracene. *Cancer Lett.* **70**, 7−14.

(30) Luch, A., Schober, W., Greim, H., Doehmer, J., Jacob, J., Seidel, A., and Baird, W. M. (2000) Metabolic activation of dibenzo[*a*,*l*]-pyrene by cytochrome P450 enzymes to stable DNA adducts occurs exclusively through the formation of the (−)-*trans*-(11*R*,-12*R*)-diol. *Polycyclic Aromat. Compds.* **21**, 87−98.

(31) Ralston, S. L., Lau, H. H. S., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1994) The potent carcinogen dibenzo[*a*,*l*]pyrene is metabolically activated to fjord-region 11,12-diol-13,14-epoxides in human mammary carcinoma MCF-7 cell cultures. *Cancer Res.* **54**, 887−890.

(32) Ralston, S. L., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1995) Stereoselective activation of dibenzo[*a*,*l*]pyrene to (−)-*anti*(11*R*,12*S*,13*S*,14*R*)- and (+)-*syn*(11*S*,12*R*,13*S*,14*R*)-11,12-diol-13,14-epoxides which bind extensively to deoxyadenosine residues of DNA in the human mammary carcinoma cell line MCF-7. *Carcinogenesis* **16**, 2899−2907.

(33) Luch, A., Friesel, H., Seidel, A., and Platt, K. L. (1998) Tumor-initiating activity of the (+)−(*S*,*S*)- and (−)-(*R*,*R*)-enantiomers of *trans*-11,12-dihydroxy-11,12-dihydrodibenzo[*a*,*l*]pyrene in mouse skin. *Cancer Lett.* **136**, 199−128.

(34) Ralston, S. L., Lau, H. H. S., Seidel., A., Luch, A., Platt, K. L., and Baird, W. M. (1994) Identification of dibenzo[*a*,*l*]pyrene-DNA adducts formed in cells in culture and in mouse skin. *Polycyclic Aromat. Compds.* **6**, 199−206.

(35) Gonzalez, F. J., and Kimura, S. (1999) Role of gene knockout mice in understanding the mechanisms of chemical toxicity and carcinogenesis. *Cancer Lett.* **143**, 199−204.

(36) Gonzalez, F. J. (2001) The use of gene knockout mice to unravel the mechanisms of toxicity and chemical carcinogenesis. *Toxicol. Lett.* **120**, 199−208.

(37) Buters, J. T. M., Sakai, S., Richter, T., Pineau, T., Alexander, D. L., Savas, U., Doehmer, J., Ward, J. M., Jefcoate, C. R., and Gonzalez, F. J. (1999) Cytochrome P450 CYP1B1 determines susceptibility to 7,12-dimethylbenz[*a*]anthracene-induced lymphomas. *Proc. Natl. Acad. Sci. U.S.A.* **96**, 1977−1982.

(38) Schober, W. (2000) Ph.D. Thesis, Technical University of Munich, Germany.

(39) Savas, U., and Jefcoate, C. R. (1994) Dual regulation of cytochrome P450EF expression via the aryl hydrocarbon receptor and protein stabilization in C3H/10T1/2 cells. *Mol. Pharmacol.* **45**, 1153−1159.

(40) Masuda, Y., and Kagawa, R. (1972) A novel synthesis and carcinogenicity of dibenzo[*a*,*l*]pyrene. *Chem. Pharm. Bull.* **20**, 2736−2737.

(41) Prahalad, A. K., Ross, J. A., Nelson, G. B., Roop, B. C., King, L. C., Nesnow, S., and Mass, M. J. (1997) Dibenzo[*a*,*l*]pyrene-induced DNA adduction, tumorigenicity, and Ki-*ras* oncogene mutations in strain A/J mouse lung. *Carcinogenesis* **18**, 1955−1963.

(42) De Raat, W. K., Kooijman, S. A. L. M., and Gielen, J. W. J. (1987) Concentrations of polycyclic hydrocarbons in airborne particles in Netherlands and their correlation with mutagenicity. *Sci. Total. Environ.* **66**, 95−114.

(43) Kozin, I. S., Gooijer, C., and Velthorst, N. H. (1995) Direct determination of dibenzo[*a*,*l*]pyrene in crude extracts of environmental samples by laser excited Shpol'skii spectroscopy. *Anal. Chem.* **67**, 1623−1626.

(44) Maga, J. A. (1986) Polycyclic aromatic hydrocarbon (PAH) composition of Mesquite (*Prosopis fuliflora*) smoke and grilled beef. *J. Agric. Food Chem.* **34**, 249−251.

(45) Mumford, J. L., Harris, D. B., Williams, K., Chuang, J. C., and Cooke, M. (1987) Indoor air sampling and mutagenicity studies of emissions from unvented coal combustion. *Environ. Sci. Technol.* **21**, 308−311.

(46) Mumford, J. L., Xueming, L., Fuding, H., Xu, B. L., and Chuang, J. C. (1995) Human exposure and dosimetry of polycyclic aromatic hydrocarbons in urine from Xuan Wei, China with high lung cancer mortality associated with exposure to unvented coal smoke. *Carcinogenesis* **16**, 3031−3036.

(47) Snook, M. E., Severson, R. F., Arrendale, R. F., Higman, H. C., and Chortyk, O. T. (1977) The identification of high molecular weight polynuclear aromatic hydrocarbons in a biologically active fraction of cigarette smoke condensate. *Beitr. Tabakforsch.* **9**, 79−101.

(48) Wise, S. A., Chesler, S. N., Hilpert, L. R., May, W. E., Rebbert, R. E., Vogt, C. R., Nishioka, M. G., Austin, A., and Lewtas, J. (1985) Quantification of polycyclic aromatic hydrocarbons and nitro-substituted polycyclic aromatic hydrocarbons and mutagenicity testing for the characterization of ambient air particulate matter. *Environ. Intern.* **11**, 147−160.

(49) Hoffmann, D., and Hoffmann, I. (1998) Tobacco Smoke Components. (Letter to the Editor). *Beitr. Tabakforsch.* **18**, 49−52.

(50) IARC (1983) *Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental, and Experimental Data*, IARC, Lyon, France.
(51) Dipple, A., Moschel, R. C., and Bigger, C. A. H. (1984) Polynuclear aromatic carcinogens. In *Chemical Carcinogens* (Searle, C. E., Ed.) pp 41−163, ACS Monograph 182, American Chemical Society, Washington, DC.
(52) Cavalieri, E. L., and Rogan, E. G. (1995) Central role of radical cations in metabolic acitivation of polycyclic aromatic hydrocarbons. *Xenobiotica* **25**, 677−688.
(53) Arif, J. M., Smith, W. A., and Gupta, R. C. (1997) Tissue distribution of DNA adducts in rats treated by intramammillary injection with dibenzo[*a*,*l*]pyrene, 7,12-dimethylbenz[*a*]anthracene and benzo[*a*]pyrene. *Mutat. Res.* **378**, 31−39.
(54) Arif, J. M., Smith, W. A., and Gupta, R. C. (1999) DNA adducts formation and persistence in rat tissues following exposure to the mammary carcinogen dibenzo[*a*,*l*]pyrene. *Carcinogenesis* **20**, 1147−1150.
(55) Hughes, N. C., and Phillips, D. H. (1990) Covalent binding of dibenzpyrenes and benzo[*a*]pyrene to DNA: evidence for synergistic and inhibitory interactions when applied in combination to mouse skin. *Carcinogenesis* **11**, 1611−1619.
(56) Melendez-Colon, V. J., Luch, A., Seidel, A., and Baird, W. M. (1999) Cancer initiation by polycyclic aromatic hydrocarbons results from formation of stable DNA adducts rather than apurinic sites. *Carcinogenesis* **20**, 1885−1891.
(57) Luch, A., Glatt, H.-R., Platt, K. L., Oesch, F., and Seidel, A. (1994) Synthesis and mutagenicity of the diastereomeric fjord-region 11,-12-dihydrodiol 13,14-epoxides of dibenzo[*a*,*l*]pyrene. *Carcinogenesis* **15**, 2507−2516.
(58) Seidel, A., Friedberg, T., Löllmann, B., Schwierzok, A., Funk, M., Frank, H., Holler, R., Oesch, F., and Glatt, H.-R. (1998) Detoxification of optically active bay- and fjord-region polycyclic aromatic hydrocarbon dihydrodiol epoxides by human glutathione transferase P1−1 expressed in Chinese hamster V79 cells. *Carcinogenesis* **19**, 1975−1981.
(59) Gill, H. S., Kole, P. L., Wiley, J. C., Li, K. M., Higginbotham, S., Rogan, E. G., and Cavalieri, E. L. (1994) Synthesis and tumor-initiating activity in mouse skin of dibenzo[*a*,*l*]pyrene *syn*- and *anti*-fjord-region diolepoxides. *Carcinogenesis* **15**, 2455−2460.
(60) Amin, S., Desai, D., Dai, W., Harvey, R. G., and Hecht, S. S. (1995) Tumorigenicity in newborn mice of fjord region and other sterically hindered diol epoxides of benzo[*g*]chrysene, dibenzo[*a*,*l*]-pyrene (dibenzo[*def*,*p*]chrysene), 4H-cyclopenta[*def*]chrysene and fluoranthene. *Carcinogenesis* **16**, 2813−2817.
(61) Amin, S., Krzeminski, J., Rivenson, A., Kurtzke, C., Hecht, S. S., and El-Bayoumy, K. (1995) Mammary carcinogenicity in female CD rats of fjord region diol epoxides of benzo[*c*]phenanthrene, benzo[*g*]chrysene and dibenzo[*a*,*l*]pyrene. *Carcinogenesis* **16**, 1971−1974.
(62) Crespi, C. L., Penman, B. W., Steimel, D. T., Smith, T., Yang, C. S., and Sutter, T. R. (1997) Development of a human lymphoblastoid cell line constitutively expressing human CYP1B1 cDNA: substrate specificity with model substrates and promutagens. *Mutagenesis* **12**, 83−89.
(63) Guengerich, F. P. (1992) Metabolic activation of carcinogens. *Pharmacol. Ther.* **54**, 17−61.
(64) Guengerich, F. P., and Shimada, T. (1998) Activation of procarcinogens by human cytochrome P450 enzymes. *Mutat. Res.* **400**, 201−213.
(65) Christou, M., Savas, U., Schroeder, S., Shen, X., Thompson, T., Gould, M. N., and Jefcoate, C. R. (1995) Cytochromes CYP1A1 and CYP1B1 in the rat mammary gland: cell-specific expression and regulation by polycyclic aromatic hydrocarbons and hormones. *Mol. Cell. Endocrin.* **115**, 41−50.
(66) Kimura, S., Gonzalez, F. J., and Nebert, D. W. (1986) Tissue-specific expression of the mouse dioxin-inducible P(1)450 and P(3)-450 genes: differential transcriptional activation and mRNA stability in liver and extrahepatic tissues. *Mol. Cell. Biol.* **6**, 1471−1477.
(67) Alexander, D. L., Eltom, S. E., and Jefcoate, C. R. (1997) Ah receptor regulation of CYP1B1 expression in primary mouse embryo-derived cells. *Cancer Res.* **57**, 4498−4506.
(68) Shen, Z., Liu, J., Wells, R. L., and Elkind, M. M. (1994) cDNA cloning, sequence analysis, and induction by aryl hydrocarbons of a murine cytochrome P450 gene, Cyp1b1. *DNA Cell Biol.* **13**, 763−769.
(69) Luch, A., Coffing, S. L., Seidel, A., Greim, H., Baird, W. M., and Doehmer, J. (1999) DNA modification induced after metabolic activation of the potent carcinogen dibenzo[*a*,*l*]pyrene in V79 Chinese hamster cells stably expressing single cytochromes P450. *Polycyclic Aromat. Compds.* **16**, 71−78.
(70) Luch, A., Schober, W., Soballa, V. J., Raab, G., Greim, H., Jacob, J., Doehmer, J., and Seidel, A. (1999) Metabolic activation of dibenzo[*a*,*l*]pyrene by human cytochrome P450 1A1 and P450 1B1 expressed in V79 Chinese hamster cells. *Chem. Res. Toxicol.* **12**, 353−364.
(71) Luch, A., Seidel, A., Glatt, H. R., Oesch, F., and Platt, K. L. (1996) Correlation of the extent of fjord-region oxidation with DNA binding and mutagenicity of the enantiomeric 11,12-dihydrodiols of dibenzo[*a*,*l*]pyrene. *Polycyclic Aromat. Compds.* **10**, 101−108.
(72) Luch, A., Seidel, A., Glatt, H.-R., and Platt, K. L. (1997) Metabolic activation of the (+)-*S*,*S*- and (−)-*R*,*R*-enantiomers of *trans*-11,-12-dihydroxy-11,12-dihydrodibenzo[*a*,*l*]pyrene: stereoselectivity, DNA adduct formation, and mutagenicity in Chinese hamster V79 cells. *Chem. Res. Toxicol.* **10**, 1161−1170.
(73) Ralston, S. L., Coffing, S. L., Seidel, A., Luch, A., Platt, K. L., and Baird, W. M. (1997) Stereoselective activation of dibenzo-[*a*,*l*]pyrene and its *trans*-11,12-dihydrodiol to fjord region 11,12-diol 13,14-epoxides in a human mammary carcinoma MCF-7 cell-mediated V79 cell mutation assay. *Chem. Res. Toxicol.* **10**, 687−693.
(74) Walker, N., Gastel, J. A., Costa, L. T., Clark, G. C., Lucier, G. W., and Sutter, T. (1995) Rat CYP1B1: an adrenal cytochrome P450 that exhibits sex-dependent expression in livers and kidneys of TCDD-treated animals. *Carcinogenesis* **16**, 1319−1327.
(75) Tang, Y. M., Wo, Y.-Y. P., Stewart, J., Hawkins, A. L., Griffin, C. A., Sutter, T. R., and Greenlee, W. F. (1996) Isolation and characterization of the human cytochrome P450 CYP1B1 gene. *J. Biol. Chem.* **271**, 28324−28330.
(76) Baron, J. M., Zwadlo-Klarwasser, G., Jugert, F., Hamann, W., Rubben, A., Mukhtar, H., and Merk, H. F. (1998) Cytochrome P450 1B1: a major P450 isoenzyme in human blood monocytes and macrophage subsets. *Biochem. Pharmacol.* **56**, 1105−1110.
(77) Baron, J. M., Holler, D., Schiffer, R., Frankenberg, S., Neis, M., Merk, H. F., and Jugert, F. K. (2001) Expression of multiple cytochrome P450 enzymes and multidrug resistance-associated transport proteins in human skin keratinocytes. *J. Invest. Dermatol.* **116**, 541−548.
(78) Heidel, S. M., MacWilliams, P. S., Baird, W. M., Dashwood, M., Buters, J. T. M., Gonzalez, F. J., Larsen, M. C., Czuprynski, C. J., and Jefcoate, C. R. (2000) Cytochrome P4501B1 mediates induction of bone marrow cytotoxicity and preleukemia cells in mice treated with 7,12-dimethylbenz[*a*]anthracene. *Cancer Res.* **60**, 3454−3460.
(79) Eltom, S. E., Larsen, M. C., and Jefcoate, C. R. (1998) Expression of CYP1B1 but not CYP1A1 by primary cultured human mammary stromal fibroblasts constitutively and in response to dioxin exposure: role of the Ah receptor. *Carcinogenesis* **19**, 1437−1444.
(80) Savas, U., Christou, M., and Jefcoate, C. R. (1993) Mouse endometrium stromal cells express a polycyclic aromatic hydrocarbon-inducible cytochrome P450 that closely resembles the novel P450 in mouse embryo fibroblasts (P450EF). *Carcinogenesis* **14**, 2013−2018.
(81) Stack, D. E., Cavalieri, E. L., and Rogan, E. G. (1998) Catecholestrogens as procarcinogens: depurinating adducts and tumor initiation. *Adv. Pharmacol.* **42**, 833−836.
(82) Cavalieri, E. L., Stack, D. E., Devanesan, P. D., Todorovic, R., Dwivedy, I., Higginbotham, S., Johansson, S. L., Patil, K. D., Gross, M. L., Gooden, J. K., Ramanathan, R., Cerny, R. L., and Rogan, E. G. (1997) Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10937−10942.
(83) Badawi, A. F., Cavalieri, E. L., and Rogan, E. G. (2001) Role of human cytochrome P450 1A1, 1A2, 1B1, and 3A4 in the 2-, 4-, and 16-alpha-hydroxylation of 17beta-estradiol. *Metabolism* **50**, 1001−1003.

TX020017Q