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Induction and Developmental Expression of Cytochrome P450IA1 Messenger RNA in Rat and Human Tissues: Detection by the Polymerase Chain Reaction[1]

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ABSTRACT

The expression of cytochrome P450 genes directly within target cells is an important determinant of human susceptibility to cancers, birth defects, and other chemically initiated diseases. One pivotal gene, *CYP1A1*, codes for an inducible cytochrome P450 isozyme (P450IA1) responsible for the bioactivation of numerous carcinogenic polycyclic hydrocarbons and aromatic amines. In this study, we used the polymerase chain reaction (PCR) to amplify endogenous P450IA1 mRNA transcripts in a variety of human and rat tissues from different stages of development. The PCR approach greatly enhanced detection sensitivities over those previously achieved and permitted characterization of constitutive as well as induced P450IA1 mRNA expression patterns in specific cell types and organs and during early gestational stages. P450IA1 mRNAs are expressed constitutively in the rat as early as day 15 of fetal liver development and increased in level with increasing developmental age. Transplacental treatment of fetal rats with 3-methylcholanthrene resulted in marked increases in P450IA1 mRNA levels, and responsiveness to the inducer also increased in concordance with developmental age. Comparatively lower constitutive and induced levels of the mRNA were detected in rat lung and kidney, but no P450IA1 mRNA was detected in the rat testis. PCR results obtained from experiments with human tissue samples demonstrated the presence of P450IA1 transcripts in whole organs, in purified cell fractions (lymphocytes, macrophages), and in fetuses as early as day 45 of human gestation. Data from cell culture studies indicated markedly higher levels of P450IA1 PCR product in human pulmonary alveolar macrophages following treatment with the inducing agent β-naphthoflavone. These results underscore the potential role of P450IA1 as a key determinant of individual susceptibility to tissue-specific and developmentally related cancers associated with certain environmental chemical exposures.

INTRODUCTION

The cytochrome P450 superfamily of genes code for an array of enzymes catalyzing the mixed function oxidation of a wide variety of environmental chemicals including proximate mutagens, carcinogens, and teratogens (1, 2). Nucleic acid hybridization techniques, immunoassays, and substrate probes have all proved valuable in characterizing P450[2] genes and their respective products. However, extensive sequence relatedness within certain P450 subfamilies, ranging to 98%, together with associated overlapping substrate specificities, and low levels of expression in certain tissues, has made it difficult to detect and discriminate between highly similar P450 gene products.

A number of P450s are expressed constitutively in the liver of adult animals and hepatic levels of certain subfamily members are induced markedly in response to chemical exposures (1–3). It is clear, however, that extrahepatic organs, *e.g.*, the lung, are frequent target sites for cancers initiated by chemicals requiring bioactivation (1, 3, 4). Some P450 genes also are expressed in cells of extrahepatic organs, but typically at much lower levels (2, 4). Due in part to difficulties associated with procurement of human tissues and technical analyses of low levels of highly related P450 products, P450 expression patterns and factors controlling P450 gene regulation in extrahepatic and fetal tissues have been investigated less extensively.

Organisms in early stages of development are exquisitely sensitive to the teratogenic effects of chemical exposures (5, 6). Levels of biotransformation activities are normally much lower in mammalian tissues from early gestational ages in comparison to those observed in adults (5–7). Yet, a number of chemicals regarded as possessing high teratogenic potential in humans (*e.g.*, valproic acid, phenytoin, warfarin, cigarette smoke constituents) are known to undergo bioactivation by cytochrome P450 isozymes (5–7). The involvement of the P450 system in developmentally related cancers also has been demonstrated in mouse experiments investigating the aryl hydrocarbon-responsive (*Ah*) genotype (7, 8), which classically has been assessed by P450-mediated aryl hydrocarbon hydroxylase activity. *Ah*-responsive mice have been shown to be at higher risk for the development of liver and lung cancers subsequent to polycyclic hydrocarbon exposure *in utero* (7, 8). Despite their critical roles in directing the toxicological fate of exposed substances, analyses of specific P450 products in tissues of early developing animals have been difficult due to limiting amounts of tissue and low levels of product.

One human cytochrome P450 subfamily member, P450IA1, as well as its orthologue in other mammalian species, is highly inducible following exposure to certain dioxins and polycyclic hydrocarbons (2, 7). *Ah*-responsive genotype correlates highly with the capacity to induce P450IA1 expression (2, 7). The P450IA1 isozyme has been demonstrated to be a key participant in the bioactivation of a number of procarcinogenic substances, including aromatic amines and other prevalent environmental hydrocarbons (2, 3, 7). Benzo(a)pyrene, *e.g.*, is metabolized in part by P450IA1 to its ultimate carcinogenic intermediate, the *trans*-7,8-diol,9-10-epoxide moiety (3). Clearly, the regulation of P450IA1 gene expression in cells, in particular those exposed directly to environmental substances, such as pulmonary alveolar macrophages or lung epithelial cells, may be a prime determinant of susceptibility to the detrimental effects of chemical exposure.

Previous reports also have suggested that the induction of P450IA1 expression in humans may be subject to genetic polymorphism, such that the most responsive members of the population may be at highest risk for the development of lung cancers (7, 9). In this regard, levels of induced P450IA1 mRNA in mitogen-stimulated human lymphocyte preparations have been reported to accurately reflect aryl hydrocarbon hydroxylase activities measured in the same preparations (9).

In this report, we utilize the polymerase chain reaction (PCR) along with highly discriminating oligomer primers to assess the adult and fetal expression of P450IA1 mRNAs in humans and in a rat animal model. The analyses permitted the evaluation

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[2] The abbreviations used are: P450, cytochrome P-450; PCR, polymerase chain reaction; MC, 3-methylcholanthrene; cDNA, complementary DNA; poly(A)+ RNA, polyadenylated RNA.

of P450IA1 mRNAs at sensitivities greatly exceeding those previously obtained. This is the first report detailing constitutive levels of P450IA1 mRNA expression in adult human lymphocytes, pulmonary alveolar macrophages, or lung tissues. Similarly, the procedures enabled the first clear demonstration of constitutive P450IA1 mRNA expression in fetal tissue preparations obtained from both humans and rats. In addition, we show that polycyclic hydrocarbon pretreatment of human macrophages *in vitro*, or developing rats *in utero*, results in marked elevation of P450IA1 mRNA levels.

MATERIALS AND METHODS

Tissue Sources and RNA Preparation. Rats (Sprague-Dawley) were obtained from Tyler Laboratories (Bellevue, WA). Rat fetuses were obtained from time-mated pregnant animals; the day following mating designated as day 0 of gestation. The gestational period of a rat is typically 22 days. The developmental ages referenced in the text are measured from day 0 of gestation, such that a day 29 rat is a 1-week neonate, etc. All neonatal and adult rats were male. Pretreatments with inducing agents, tissue preparation, and RNA isolation were performed as described previously (10, 11). For MC induction studies, rats received 40 mg/kg MC dissolved in corn oil by i.p. injection 48 h and again at 16 h before sacrifice. To assess prenatal time points, hepatic tissues from at least 10 fetuses from 2–3 pregnant rats were pooled for RNA extraction.

All human tissue specimens were acquired and utilized in accordance with approvals obtained from the University of Washington's human subject review committee. Human fetal tissues were obtained subsequent to dilatation and curettage procedures and acquired through the Laboratory of Central Human Embryology at the University of Washington. Gestational age was estimated by crown-rump length. Human adult tissue samples were obtained from either volunteers (lymphocytes) or from surgical or organ donor patients at local area hospitals. Lymphocytes were separated from whole blood by centrifugation in Leucoprep tubes (Becton/Dickinson). Human pulmonary alveolar macrophages were obtained by whole lung bronchoalveolar lavage with sterile 0.9% sodium chloride. The recovered lavage fluid was pooled, filtered through moistened gauze, and centrifuged for 10 min at 600 × *g*. Cell pellets were washed twice in Hanks' balanced salt solution and suspended at 10^6 viable alveolar macrophages per ml in medium consisting of 50% NCTC 135/50% Ham's F-10 (Flow Laboratories) with 5% calf serum supreme (Gibco) and supplemented with penicillin (100 units/ml) and streptomycin (100 μg/ml). The cells acquired from lavage typically contained greater than 80% alveolar macrophages with macrophage viability greater than 85%. Tissue samples were quick-frozen immediately upon procurement and stored either in liquid nitrogen or at −80°C until use. RNA was extracted from human tissues according to the method of Chomczynski and Sacchi (12), and RNA concentrations were quantified by absorbance at 260 nm. Integrity of RNA samples and transfer efficiencies were assessed by rRNA visualization with ethidium bromide staining of gels and by hybridization of membranes to a T4 kinase ^{32}P-labeled 18S rRNA oligomer probe (see below). Total RNA was fractionated in denaturing formaldehyde gels and transferred to Genescreen Plus (NEN/DuPont) membranes as described previously (11).

cDNA Synthesis and Primer Preparation. Five μg of total RNA and 20 pmol of oligo $d(T)_{20}$ were dissolved in 12 μl of diethyl pyrocarbonate-treated water/0.1 mM EDTA, heated to 75°C for 2 min, and placed at 42°C. The samples were brought up to 20 μl volume in final buffer concentrations of 25 mM KCl, 6 mM $MgCl_2$, 10 mM dithiothreitol, 0.25 mM concentrations of all four deoxynucleotide triphosphates, 50 mM Tris-Cl (pH 8.3), 25 units of placental RNase inhibitor (Amersham), and 17 units of avian myeloblastosis virus reverse transcriptase (Seikagaku). Samples were then incubated at 42°C for 1 h. In other experiments, the antisense PCR primers were used for cDNA synthesis in place of oligo $d(T)_{20}$; however, this substitution had no effect on the final results obtained with the PCR studies.

Oligomers were synthesized with an Applied Biosystems 380A synthesizer by the Howard Hughes Medical Institute at the University of Washington. The rat and human P450IA1 sequences utilized for forward (FP) and reverse (RP) PCR primers, and for the Southern blot hybridization probes (HP), were as follows:

Rat primers

IA1FP, 5′-CCATGACCAGGAACTATGGG-3;
IA1RP, 5′-TCTGGTGAGCATCCAGGACA-3′;
IA1HP, 5′-AGCCTGGAGATGCTGAGGAC-3′;

Human primers

IA1FP, 5′-TAGACACTGATCTGGCTGCAG-3′;
IA1RP, 5′-GGGAAGGCTCCATCAGCATC-3′; and
IA1HP, 5′-CAGGCAGGATCCCTTAGGCT-3′

The rat primers were targeted to sites in the cDNA flanking the intron/exon boundary of the corresponding genomic regions of exons 6–7 and were predicted to produce a 341-base pair product following amplification of the P450IA1 cDNA. The human primers were targeted to sites in the cDNA within the corresponding genomic exon 7 and were expected to produce a cDNA product of 146 bp. The rat/human 18S rRNA antisense probe, 5′-CACCTCTAGCGGCGCAATAC-3′, was utilized in hybridization experiments to monitor RNA integrity and efficiency of gel transfer (data not presented).

PCR Reactions. PCR reactions were performed essentially as described previously (13), with the following modifications. Reactions were conducted in a final volume of 50 μl consisting of 25 mM Tris-Cl (pH 8.3), 50 mM KCl, 3 mM $MgCl_2$, 0.2 mM concentrations of each deoxynucleotide triphosphate, 0.1 mg/ml of bovine serum albumin, 20 pmol each of forward and reverse primer, 2 μl of cDNA, and 2 units of Taq DNA polymerase (New England Biolabs). After the addition of 3 drops of light mineral oil (Sigma), the reactions were heated to 93°C for 4 min and then immediately cycled 30 times through a 1-min denaturation step at 93°C, a 1.5-min annealing step at 54°C, and a 1-min extension step at 72°C. Ten μl of the aqueous phase were separated through a mixture of 2% NuSieve/1% SeaKem LE agarose (FMC) and visualized by ethidium bromide staining. Southern transfer to Genescreen Plus membrane was achieved with capillary blotting. To verify identity of the PCR products, the Southern-blotted membranes were probed with independent ^{32}P-labeled hybridization oligomers, targeted to expected internal sequence within the PCR products. The resulting blots were subjected to autoradiography with Kodak XAR film, and the sizes of the products agreed with those expected for the amplified region of the cDNAs. PCR amplifications and Southern blot analyses were conducted at least twice to verify the reproducibility of results. In separate experiments (data not presented), the identity of PCR products also was established by DNA sequence analysis.

Cell Culture Conditions. The resuspended pulmonary macrophages (see above) were plated in 75-cm^2 flasks and incubated at 37°C in humidified 5% CO and air. Plated cells were allowed to adhere for 12 h and then were incubated in fresh media (see above) containing various concentrations of β-naphthoflavone (0, 1, 3, and 6 μg/ml) for 24 h. Cells were then washed in Hanks' balanced salt solution and total RNA was isolated as described (12).

RESULTS

Rat P450IA1 Gene Expression. Fig. 1 presents data obtained from Northern blot analysis of rat P450IA1 expression in liver tissue as a function of development in untreated, phenobarbital-treated, and MC-treated animals. With the highly specific oligomer probe used, no detectable P450IA1 mRNA was apparent in any of the untreated or phenobarbital-treated liver specimens. However, in livers from MC-induced rats 29, 46, and 64 days of age (day 0 of gestation was designated as the day after mating; the animals were born on day 22), a single band of the expected size for P450IA1 mRNA (2.6 kilobases) was clearly apparent. In separate experiments we also detected very low

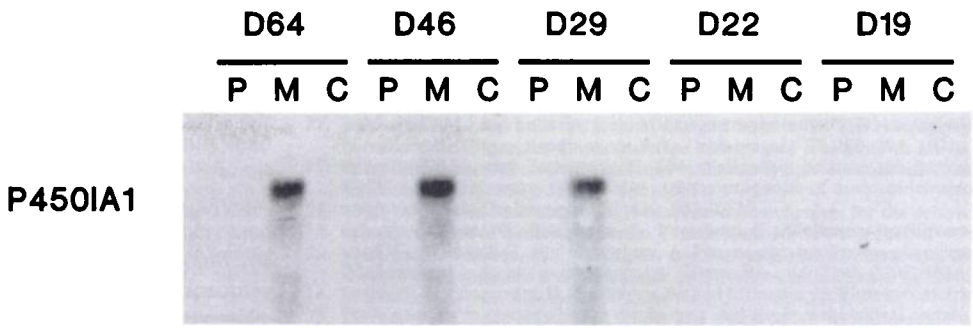

Fig. 1. Developmental Northern blot of total rat liver RNA probed for P450IA1. Total RNA (25 μg/lane) was analyzed for P450IA1 mRNA content after isolation from uninduced (*C*), 3-methylcholanthrene-treated (*M*), or phenobarbital-treated (*P*) liver, from rats 64, 46, 29, 22, or 19 days (*D*) of age. The visible band corresponded to a molecular size of 2.6 kilobases, in agreement with the expected size for rat P450IA1 mRNA.

levels of P450IA1 mRNA in MC-treated fetal liver at day 22 of gestation, but only after probing 20 μg of poly(A) + RNA per sample lane and 5-day autoradiographic exposures (data not shown). In previous studies, we ascertained, through comparisons of results with solution hybridization experiments *versus* Northern blots, that our Northern blot analyses are capable of detecting mRNA present in tissues when expressed at an average of approximately 2–3 copies/cell (11, 14). This assumes a relatively homogeneous distribution of expression within cells comprising the organ, *e.g.*, liver. Therefore, the absence of detectable bands in Fig. 1 indicated expression levels below this threshold.

We examined whether a PCR-based assay for mRNA expression could provide enhanced detection sensitivity while retaining the requisite high level of selectivity. cDNA was synthesized from RNA fractions purified from rat liver and the P450IA1 components were amplified with PCR forward and reverse primer sets (see "Materials and Methods" for details).

The results shown in Fig. 2 illustrate the PCR products obtained with MC-treated rat liver from three developmental ages. Fig. 2*A* shows the results of ethidium bromide staining of an agarose gel containing the PCR products (*Lanes 1–3*), relative to the DNA size markers (*Lane M*) and a negative control (*Lane 4*). The negative controls used throughout these studies consisted of PCR reactions performed with RNA samples to which no reverse transcriptase was added. The absence of detectable signal in these controls provided evidence that the PCR products obtained in the sample lanes were the direct result of cDNA amplification. A PCR product of 341 base pairs, the predicted size for rat P450IA1, was clearly visible in *Lanes 1–3*. The relative amount of product in *Lane 1*, from the day 29 animals, was visibly less than that present in the day 46 or day 64 samples. Fig. 2*B* presents the same gel, after Southern transfer and hybridization to a labeled oligomer probe designed to recognize sequence internal to the PCR primer sites. Only *Lanes 1–3* showed evidence of signal, and the bands observed were in direct concordance with those seen in Fig. 2*A*. These data indicated that the PCR-based assay was capable of selectively amplifying rat P450IA1 cDNA.

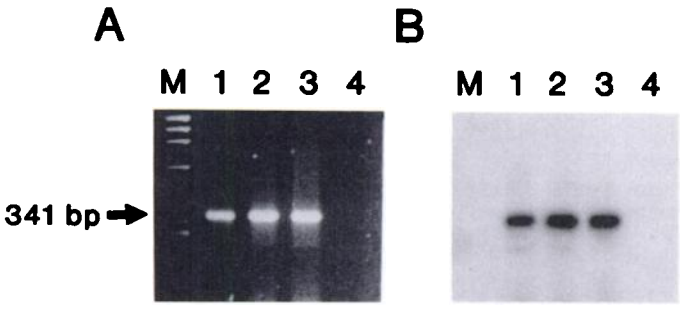

Fig. 2. Rat P450IA1 PCR products. *A*, ethidium bromide-stained agarose gel containing PCR products resulting from amplification of rat liver P450IA1 cDNA. *Lane M* contains DNA size markers; *Lane 1*, day 29; *Lane 2*, day 46; *Lane 3*, day 64; *Lane 4*, negative control consisting of a RNA sample amplified directly, without prior reverse transcription. *B*, same gel as in *A* following Southern blotting and hybridization to an internal P450IA1 oligomer probe. The visible band was 341 base pairs (*bp*) long, as predicted from the positions of the PCR primers.

The data presented in Fig. 3 enable comparison between the relative sensitivity of the PCR technique with that of Northern blotting. P450IA1 mRNA expression clearly was apparent with PCR analysis (Fig. 3*A*), even in RNA samples previously demonstrating no signal upon Northern blot hybridization (Fig. 1). P450IA1 gene expression was detectable with PCR in control livers from day 15 fetuses (day 15 of gestation was the earliest developmental time where livers were macroscopically recognizable thus allowing their dissection free of neighboring tissues). It should be noted that the day 15 data of Fig. 3*A* were the result of a long autoradiographic exposure, such that the counterpart data on the remaining portions of the figure were thoroughly blackened and unrecognizable with the equivalent exposure. This observation demonstrated the much lower levels of P450IA1 mRNA present at day 15 relative to even the next time point (day 19) assessed. At each developmental stage, higher levels of P450IA1 PCR product were apparent in the MC-treated samples compared to the controls. Low levels of P450IA1 mRNA are also detected in the untreated samples. Fig. 3*A*, *Lane B*, is a negative control lane, similar to that described for Fig. 2. These results indicated that the PCR-based assay offered a powerful enhancement in sensitivity over more conventional means of mRNA analyses.

To extend these observations, we used the PCR technique with RNAs purified from rat extrahepatic tissues. Fig. 3*B* presents data obtained with lung and kidney tissue obtained from untreated and MC-treated day 22 (fetal) and day 29 (neonatal) animals. Higher levels of the 341-base pair P450IA1 PCR product were clearly evident in MC treated day 29 lung and kidney when compared to controls. Regardless of pretreatment, day 22 fetuses exhibited only traces of P450IA1 expression in the lung or kidney, apparent only after long autoradiographic exposure.

Fig. 3*C* presents the results obtained upon Southern blotting P450IA1 PCR products from day 46 rats. Higher levels of PCR product were evident in MC-treated animals *versus* controls in liver, lung, and kidney. We reproducibly were unable to detect P450IA1 in rat testis, even from induced animals and after prolonged periods of autoradiography. Amplification reactions also were performed with testes poly(A+) RNA fractions isolated from day 64 Sprague-Dawley rats, but still we observed no evidence of expression of this gene (data not shown). As a positive control, the same testes RNA samples were assessed for P450IIB1 PCR products, which were readily apparent (data not presented). Of the tissues studied, the lack of P450IA1

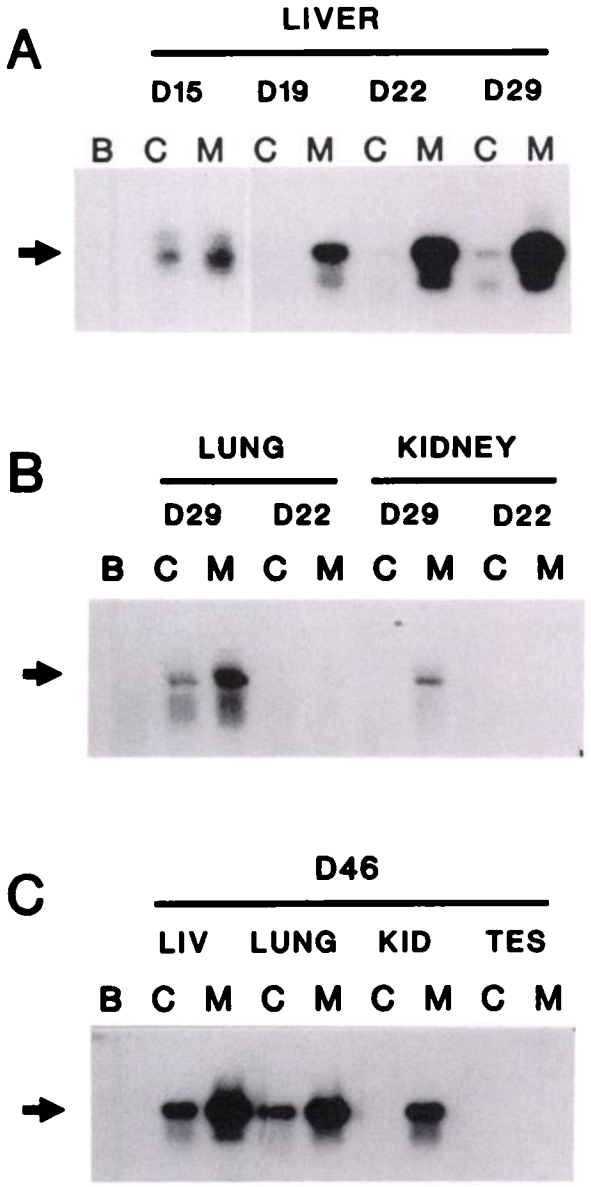

Fig. 3. Southern blot of PCR products obtained from rat tissues during development. Untreated animals and MC-treated animals are designated by *C* or *M*, respectively. *Lane B*, negative controls. In all cases, the *arrow* to the *left* of each panel corresponds to a molecular size of 341 base pairs. *A* contains PCR products from fetal [day (*D*) 15, 19, and 22 time points] and neonatal [day 29] rat livers. The portion of *A* from day 15 was exposed approximately 8 times longer (4 h *versus* 30 min) than the remaining portion of the panel. *B* was taken from an experiment using fetal (day 22) and neonatal (day 29) lung and kidney as tissue sources for PCR analysis. *C* represents liver, lung, kidney, and testes samples obtained from rats 46 days of age.

expression therefore appeared unique to the testis. P450IA1 PCR products were not detected in untreated kidney but were clearly present in kidneys from MC-induced rats.

The data presented for P450IA1 expression provide evidence for a correlation between the respective levels of P450IA1 PCR product and P450IA1 mRNA. This was apparent in comparing results of PCR data from Fig. 3 with the Northern blot data of Fig. 1. Likewise, following animal pretreatment with MC, a well-characterized activator of P450IA1 gene transcription, increased levels of P450IA1 PCR product clearly were apparent in liver throughout all stages of development (Fig. 3*A*) and in lung and kidney from day 29 or 46 animals (Fig. 3, *B* and *C*).

Human P450IA1 Gene Expression. In the course of our investigations, we were unable to routinely detect human P450IA1 mRNA expression with Northern or slot blot analyses in tissue or cell samples obtained from normal human subjects. Illustrating these results, Fig. 4 presents a Northern blot probed with a ^{32}P-labeled oligomer specific for human P450IA1 mRNA (similar data were also obtained with a human P450IA1 cDNA probe). The pulmonary macrophages utilized in *Lanes 1–4* were obtained by bronchoalveolar lavage from four subjects. RNAs in *Lanes 5–8* were isolated from lung surgical samples obtained from four different individuals. A positive control lane consisting of RNA isolated from β-naphthoflavone-induced human HepG2 hepatoma cells was included in *Lane 9* of the Northern blot which demonstrated a strong signal of the expected size for human P450IA1 mRNA (2.6 kilobases). Northern blotting experiments were also undertaken to evaluate P450IA1 mRNA expression in various human fetal tissues, but in these samples as well, we were unable to detect the corresponding RNA (data not shown).

We therefore used the PCR assay using the human P450IA1 forward and reverse primer sets, as well as the internal hybridization oligomer probe, to assess P450IA1 mRNA expression in selected human tissues and cells. Fig. 5 illustrates results of PCR experiments performed with RNAs isolated from human lung, lymphocytes, and pulmonary alveolar macrophages. The data from Fig. 5*A* demonstrate that in each sample assessed from 7 different individuals, P450IA1 PCR product was detected. *Lane 1* represented a negative control. Similarly in Fig. 5*B*, macrophage (*Lanes 2* and *3*) and lung (*Lane 3*) samples from three individuals examined by Northern blot in Fig. 5 demonstrated clear evidence of P450IA1 mRNA expression.

Expression of P450IA1 in human fetal tissues also was detected by PCR analysis. The data presented in Fig. 6 demonstrate the presence of the expected 146-base pair PCR product in fetal tissue as early as day 45 of gestation (the earliest time that we have been able to obtain tissue). Expression levels of P450IA1 in human fetal kidney (Fig. 6*A*, *Lanes 2* and *3*) were quite low in available samples relative to the other tissues examined, similar to our findings with fetal rats (Fig. 3). In the fetal adrenal (Fig. 6*A*, *Lanes 4–6*), levels of P450IA1 expression

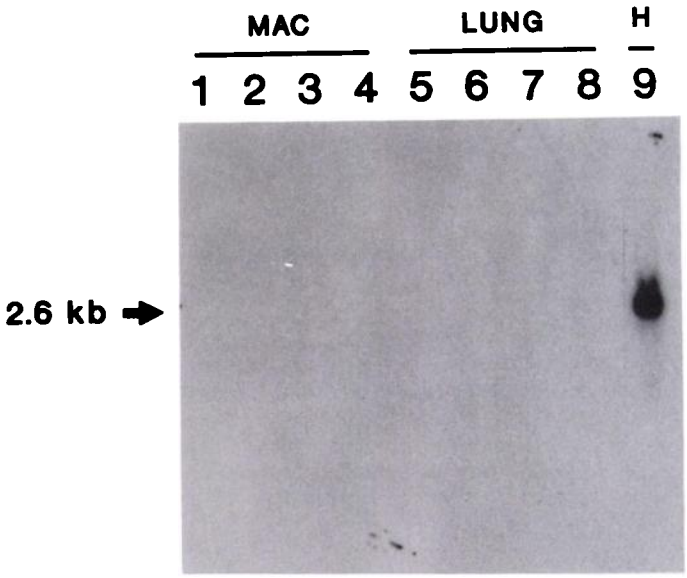

Fig. 4. P450IA1 Northern blot of human macrophages, lungs, and HepG2 hepatoma cells. Total RNA (25 μg) was analyzed from 8 different individuals: 4 pulmonary alveolar macrophages (*Lanes 1–4*); and 4 surgically obtained lung samples (*Lanes 5–8*). *Lane 9* contains RNA isolated from a β-naphthoflavone-induced human hepatoma HepG2 cell culture. The size of the expected 2.6 kilobase (*kb*) P450IA1 mRNA is indicated.

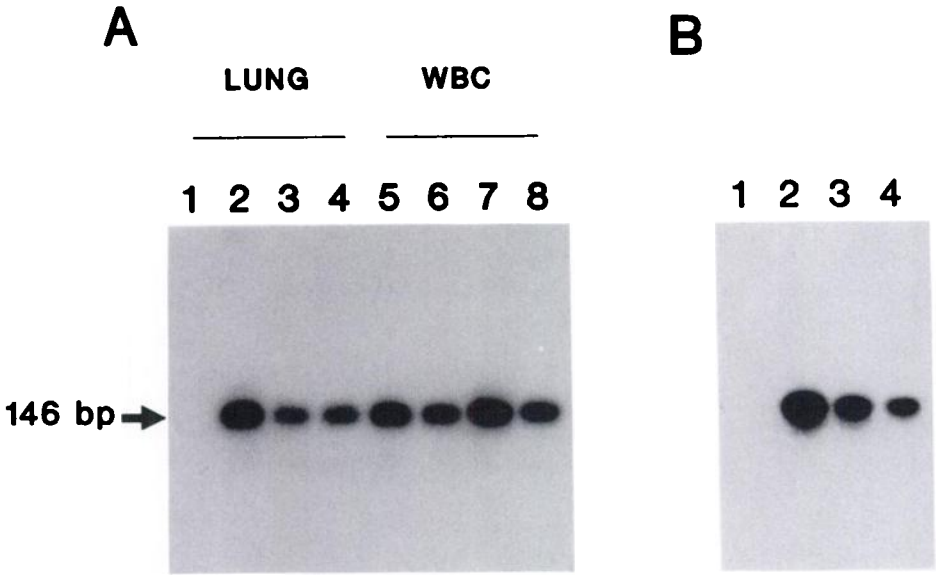

Fig. 5. Southern blot of PCR products obtained from adult human tissues. In *A* and *B* the 146-base pair (*bp*) P450IA1 cDNA products are visualized following Southern blotting and hybridization of the membranes to an internal oligomer probe. *A: Lane 1*, negative control; *Lanes 2–4*, lung cDNA samples; *Lanes 5–8*, blood lymphocyte cDNA samples. *B: Lane 1*, negative control; *Lanes 2* and *3*, macrophage cDNAs; *Lane 4*, lung cDNA.

as assessed by PCR appeared to decrease with increasing fetal age. In fetal lung (Fig. 6*B*, *Lanes 2–6*) no age-related trends were apparent. However, the two oldest liver specimens in our survey, from day 70 and 85 of gestation, respectively (Fig. 6*C*, *Lanes 4* and *5*), exhibited much greater levels of product than the 45- or 57-day old tissues.

In separate experiments, we cultured freshly isolated pulmonary macrophages from a patient in the absence and in the presence of varying concentrations of the P450IA1 inducer β-naphthoflavone. We chose not to use MC in these studies since the latter substance has been reported to be toxic to cells in culture (15). The results of the PCR analyses of these samples are presented in Fig. 7. P450IA1 PCR products accumulated to strikingly higher levels in the induced preparations (*Lanes 3–5*) compared to the untreated control (*Lane 2*). *Lane 1* contained a negative control sample. All concentrations of β-naphthoflavone appeared approximately equally effective in eliciting the higher P450IA1 product levels. When total RNA extracts were examined from these cells with Northern blots, only faint traces of P450IA1 mRNA were visible in the induced samples (data not shown). These results further indicate the increased sensitivity enabled by PCR analysis.

DISCUSSION

From data reported to date, it appears that levels of P450IA1 mRNA are well correlated with the presence of functional P450IA1 enzyme (2, 9, 16). In this report, we used a sensitive PCR-based assay to enhance detection of P450IA1 gene expression. P450IA1 mRNAs were profiled in both rat and human tissues. In addition to increased sensitivity, the assay offers the advantages of simplicity and selectivity. The primers and hybridization probes and reaction conditions used enabled highly specific amplification of P450IA1 products, thus alleviating potential problems of cross-reaction as can be encountered with immunological, substrate, or cDNA-based assays (2, 14, 17, 18). Although our PCR data cannot be interpreted as being strictly quantitative, levels of PCR reaction products appeared to correlate well in our experiments with independent measures of mRNA content (*e.g.*, Northern blots; see Fig. 1) and with predicted P450IA1-inductive effects of MC and β-naphthoflavone (Figs. 3 and 7, respectively). Indeed, other recent reports also have demonstrated the feasibility of using PCR for quantitative estimations of mRNA content (19, 20).

A portion of this study focused on the rat animal model to facilitate experimental analyses of P450IA1 gene expression not practical in humans. The data obtained with the rat system permitted the following novel observations: (*a*) constitutive hepatic expression of P450IA1 mRNA occurs as early as day 15 of gestation and increases in level with increasing developmental age; (*b*) postpartum, constitutive expression of rat P450IA1 also occurs in the lung but is not detectable in kidney or testis; and (*c*) pretreatment with MC generally resulted in large increases in tissue levels of P450IA1 PCR product, and inducer responsiveness increased in liver, lung, and kidney with increasing developmental age. In rat fetal lung and kidney, P450IA1 expression was extremely low and difficult to detect relative to the corresponding fetal hepatic levels. The lack of detectable P450IA1 PCR product in the rat testis apparently represents an exception to the generalized nature of P450IA1 expression observed in most adult tissues following exposure to polycyclic hydrocarbon inducers.

We previously reported that P450IA1 mRNA expression in fetal rat liver was not detectable with Northern blot studies (11). However, other investigators have reported the presence of low levels of P450IA1 mRNA (21) and protein (22) in day 22 fetal rat liver. Since our report (11), we also have observed expression of this mRNA in MC-treated day 22 liver, but only after analysis of 20 μg of poly(A+) RNA.[3] It is now clear from the data presented in this report that the PCR technique offers a greatly increased threshold of detection for mRNA detection over methods used previously. The current procedure permits observation of both constitutive and induced levels of P450IA1 expression in the liver as early as day 15 of rat gestation.

Recently, *in situ* hybridization experiments were reported localizing P450IA1 mRNAs in the mouse conceptus (16). Constitutive expression of P450IA1 mRNA was not detected in that study in any tissues of the embryo proper during any point in development. Of interest, grain distributions for P450IA1 were apparent in maternal decidual tissue as early as day 5.3 of gestation (16). Cytochrome P450-dependent metabolism of phenoxazone ethers has been examined recently in day 11 rat conceptuses (23), and those data also indicated that extraembryonic tissues possess comparatively higher P450 activities

[3] C. M. Giachelli and C. J. Omiecinski, unpublished experiments.

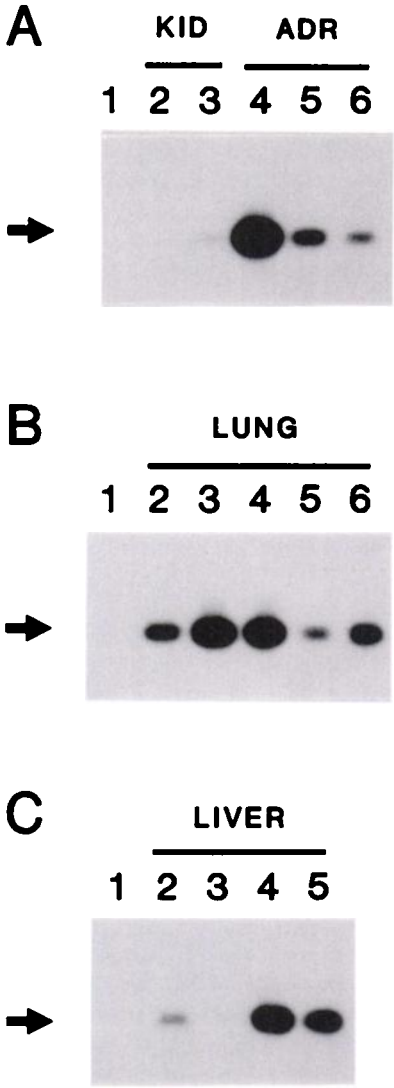

Fig. 6. Southern blot of PCR products obtained from human fetal tissues. In each panel, *Lane 1* contains negative control samples, as described for Fig. 2. *Arrows*, size of the 146-base pair P450IA1 PCR product. Approximate ages for each of the samples is given in days of gestation. *A*: *Lane 2*, 74-day kidney; *Lane 3*, 87-day kidney; *Lanes 4–6*, fetal adrenal samples of 76, 94, and 117 days, respectively. *B*: fetal lung samples; *Lane 2*, 55 days; *Lane 3*, 70 days; *Lane 4*, 101 days; *Lane 5*, 112 days; *Lane 6*, 145 days. *C*: fetal liver samples; *Lane 3*, 45 days; *Lane 4*, 57 days; *Lane 5*, 70 days; *Lane 6*, 85 days.

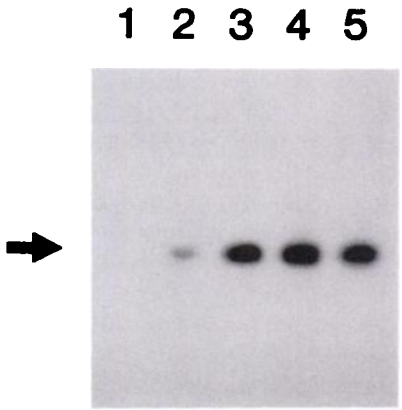

Fig. 7. Southern blot of PCR products obtained from cultured human pulmonary alveolar macrophages. *Arrow*, size of the 146 base pair P450IA1 PCR product. *Lane 1*, negative control sample. *Lane 2*, macrophage sample cultured in the absence of β-naphthoflavone. *Lanes 3–6*, samples cultured for 24 h in the presence of β-naphthoflavone, at 1, 3, and 6 μg/ml, respectively.

than the embryo itself. Previous studies with preimplanation mouse embryos, utilizing sister chromatid exchange indices (24) and benzo(a)pyrene metabolism analysis (25), have indicated the presence of polycyclic hydrocarbon-inducible P450 activities very early in mammalian development. It is likely therefore that the activities reflected in the latter studies were contributed largely by extraembryonal tissues. Our experiments with the developing rat focused on P450IA1 expression patterns in discrete fetal organs. Our results indicate that, of the organs examined, the liver is the most active fetal organ expressing P450IA1.

PCR analysis also enabled the detection of specific P450IA1 mRNAs in human tissues. Our human data demonstrate, for the first time, the constitutive nature of P450IA1 mRNA expression in a variety of adult and fetal cells and tissues. Previously, only one form of P450, P450IIIA6, has been recognized as being prominent in the human fetal liver (26). Our results indicate that the P450IA1 gene also is transcribed in fetal tissues, although at lower levels than P450IIIA6. The PCR data (Fig. 6) demonstrate expression of human P450IA1 mRNA in fetal tissues as early as day 45 of gestation, the earliest time at which we were able to obtain tissues. Significant variation in levels of P450IA1 PCR products among individual samples was apparent. The human fetal kidney preparations analyzed exhibited very low levels of expression. The fetal adrenal samples demonstrated a potentially interesting expression profile, with decreasing P450IA1 levels occurring as a function of increasing fetal age. Fetal liver samples, on the other hand, trended toward higher expression levels with increasing developmental age. In the latter respect, the liver profile appeared to follow that observed for the fetal rat (Fig. 3*A*). However, final conclusions regarding changes in P450IA1 expression levels with respect to age will require analysis of a larger number of samples. The variation in P450IA1 levels observed among fetal preparations may also reflect inherent differences among individuals or be a consequence of unknown chemical exposures. In either scenario, our results underscore the potential involvement of P450IA1-mediated metabolism of xenobiotic substances in the human conceptus.

The PCR technique further enabled the characterization of P450IA1 mRNA expression in isolated cells and tissues from adult humans. P450IA1 mRNA and protein have been detected previously in human lymphocyte preparations, but only following mitogen stimulation and treatment with polycyclic hydrocarbon inducers (9, 27). With PCR, P450IA1 mRNA expression is readily detectable from freshly isolated lymphocytes (Fig. 5). P450IA1 PCR products were also clearly apparent in samples of human lung and in pulmonary macrophages (Figs. 5 and 7). Further evidence of the quantitative nature of the PCR can be obtained from the data presented in Fig. 7, demonstrating an elevated level of P450IA1 PCR product in cells following treatment with β-naphthoflavone. These observations offer potentially exciting opportunities to explore the possibilities of using peripheral cells as bioindicators of an individual's phenotypic characteristics or as surrogate cell sources for modeling expression of biotransformation genes in target organs.

Despite apparently low levels of expression when viewed from the whole organ level, P450IA1-mediated metabolism of protoxins in these tissues may still be a significant toxicological determinant. Within complex organs, only select cells appear to be active in expressing biotransformation gene products. In the lung for example, of approximately 40 different cell types, only a few appear to express P450s. Clara cells, type II pneumocytes, pulmonary macrophages, and possibly endothelial

cells have been identified as key pulmonary cells active in the synthesis of P450 products (4, 28). In this organ, cell-specific expression patterns correlate well with the initiation of cell-specific toxicities and potentially with certain cancers (4, 29). Certain P450s also appear to be differentially inducible in discrete cell types (28).

Pulmonary macrophages are strategically localized within the alveoli and are in direct contact with inhaled chemicals and particulates (30). Our data demonstrating constitutive and induced levels of expression of P450IA1 mRNA in human macrophages (Fig. 7), together with that of others (31), argue for a potentially important role for these pulmonary phagocytic cells as a first line biotransformation system for inhaled toxins.

Studies are in progress utilizing *in situ* hybridization and immunocytochemical analyses which may aid further in identifying specific cell types in adult organs and in critical stages of human development that actively express key biotransformation enzyme genes.

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