Cytochrome P4501A1 gene polymorphism and inducibility

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Abstract

The inducibility of the enzyme CYP1A1 was examined in a cultured human lung explant system. An objective was to optimize and validate this model system for use in studies of the relationship between genotype and inducibility of CYP1A1. The enzyme was induced by the prototypic CYP1A1 inducer 2,3,7,8tetrachlorodibenzopdioxin (TCDD) and by the major tobacco smoke constituent, pyridine. The induction was observed at the level of mRNA (by RT-PCR analysis), protein (by western blot analysis), and activity (by the formation of DNA-binding derivatives from added benzo[a]pyrene (B[a]P)). In most cases, mRNA upregulation was more pronounced than protein induction. Pyridine was a better inducer than TCDD in some lung samples, whereas TCDD was a better inducer than pyridine in others. Mutant variants of the CYP1A1 gene were detected in some of the lung samples. The results show
that the explant culture system is a suitable model for examining the inducibility of human pulmonary CYP1A1, and that pyridine and TCDD may both induce human pulmonary *CYP1A1*, but by different mechanisms. The results also show that mutant alleles of the *CYP1A1* gene are present in a local population sample.

Introduction

Cytochrome P4501A1 (CYP1A1) is a member of a superfamily of enzymes involved in the biotransformation of foreign and endogenous chemicals. CYP1A1 is of toxicological importance because it catalyzes the conversion of polyaromatic hydrocarbon (PAH) constituents in tobacco smoke, such as benzo[a]pyrene, to mutagens and carcinogens (Sims and Grover, 1974). Other properties of CYP1A1 include inducibility by chemicals, including those present in tobacco smoke, and genetic polymorphism. Four genotypes of CYP1A1, resulting from various point mutations in the *CYP1A1* gene, have been characterized and designated *m1*, *m2*, *m3*, and *m4* (Cascorbi et al., 1996). Epidemiological studies have shown that

susceptibility to tobacco-induced lung cancer correlates positively with inducibility and with genotype of the enzyme (Kawajiri et al., 1990). However, it has not been established which of the genotypes most confer high inducibility. CYP1A1 inducibility studies have commonly been conducted using peripheral blood lymphocytes, and it has been argued that peripheral blood lymphocytes may not be suitable surrogates for lung tissues (Law, 1990). The present experiments were carried out, using human lung tissues, as part of an ongoing study of the relationship between genotype and inducibility of the enzyme.

Materials and methods

Lung Explant Culturing

Lung specimens were obtained from patients undergoing therapeutic lung resection for lung cancer at St. Peter's Medical Center, New Brunswick, NJ. The study protocol was approved by St. Peter's IRB committee. Each specimen was explanted and cultured as described by Stoner (1980), except that phenol red was omitted from the culture medium, CMRL-1066, because phenol red was found to impede induction in the explant model. After conditioning the explants for 18 hours, the CYP1A1 inducer pyridine (12.4 mM, final concentration) or 2,3,7,8tetrachlorodibenzo-p-dioxin (TCDD, 0.5 nM, final concentration) dissolved in dimethyl sulfoxide (DMSO), was added to the explants. Control explants were treated with DMSO only. Each treatment was in sextuplicate plates, and incubations were continued for 24 hours. Following the induction phase of the incubation, the explants were either harvested for determination of the level of CYP1A1 transcripts and protein or treated with benzo[a]pyrene (B[a]P, 20 £gM, final concentration) and incubated for another 24 hours. The B[a]P-treated tissues were then harvested for determination of *CYP1A1* activity. All incubations were at 37℃ in a 5% CO_2 chamber.

Determination of CYP1A1 mRNA

Total RNA was isolated from tissues that were harvested after incubation with pyridine, TCDD, or DMSO using TRIZOL Reagent (Gibco Life Technologies) according to the manufacturer's instructions. CYP1A1 transcripts (mRNA) in the total RNA were determined by reverse transcriptase-polymerase chain reaction (RTPCR) using Ambion's RETROscript Kit and the primers for CYP1A1 and glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH) described by Rumsby et al. (1996). *G-3-PDH* was used as a control because its expression in many mammalian tissues is high and constitutive, and is unaffected by the compounds used as inducers in the present study (Vanden Heuvel, 1997). The RT-PCR products were electrophoresed through a 3% agarose gel and visualized with ethidium bromide.

Determination of CYP1A1 Protein

Protein determination was performed by western blotting as described previously (Iba and Thomas, 1988), except that total protein rather than microsomal protein was used. Briefly, total protein was isolated from each tissue sample using TRIZOL Reagent and electrophoresed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separated proteins were blotted and probed with CD3, a mouse anti-rat P4501A1. CYP1A1 bands on the blot were then detected by enhanced chemiluminescence (ECL), using Amersham's ECL western blotting protocol. Liver microsomes from s-naphthoflavone-treated rats, in which CYP1A1 is highly induced (Iba and Thomas, 1998), were used as a positive control.

Determination of B(a)P-DNA Adducts

DNA was isolated with TRIZOL Reagent, and specific B[a]P-DNA adducts were determined by the ^{32}P-post-labeling method as described by Weyand and Wu (1995).

Determination of CYP1A1 Genotypes

Determination of CYP1A1 genotypes was performed by restriction fragment length polymorphism analysis of lung DNA using the protocol described by Cascorbi et al. (1996). Briefly, genomic DNA was extracted from tissues as described by Ghosal and Iba (1990). The extracted DNA was then hydrolyzed with the requisite restriction enzymes, and the hydrolysates were electrophoresed on agarose gel. Fragments on the gel were visualized by ethidium bromide staining.

Other Assays

Protein was determined by the method of Lowry et al. (1951), using bovine serum albumin as a standard.

Results

Effect of Inducers on CYP1A1 mRNA and Protein Expression

Almost all of the lung specimens analyzed showed the presence of either CYP1A1 transcripts, protein, or both. For each case (patient), a piece of the lung tissue that was not explanted or cultured was processed and run alongside the cultured samples for comparative purposes. RT-PCR products from samples containing CYP1A1 mRNA yielded a 146-base pair band after agarose gel electrophoresis. Different patterns of induction were observed, with pyridine being the better inducer in some samples (Fig. 1, #85, 90) and TCDD being the more potent in others (Fig. 1, #88). Western

blot analyses of the whole homogenate proteins showed similar variances in induction (Fig. 2a-c). In addition, the western blot analyses revealed the presence of CYP1A2, a related enzyme, in most of the lung samples (Fig. 2). This was an unexpected finding, as CYP1A2 is generally considered to be peculiar to the liver (Mace et al., 1998).

Effect of Inducers on B(a)P-DNA Adducts

In the few samples that have been analyzed for adduct formation thus far, induction of CYP1A1 mRNA and protein was concomitant with enhanced activation of B(a)P to metabolites capable of forming DNA adducts. The type of inducer used in culturing also seemed to be a factor in the level of adduct formation (Fig. 3).

CYP1A1 Genotypes in the Study Sample

Table 1 shows the four reported variants of the human *CYP1A1* gene and the associated restriction fragments. The variant genotypes unequivocally identified in the study include *m2* homozygote (lane 6, Fig. 4), *m2* heterozygotes (lanes 7,8, and 9, Fig. 4), *m4* homozygote (lane 13, Fig. 4), and m4 heterozygote (lane15, Fig. 4).

Discussion

Analyses of the twelve cultured samples indicated that CYP1A1 was present and/or inducible in most of the tissues. Induction was found to be more pronounced at the mRNA level than at the protein level, in that several of the samples showed induction of mRNA by pyridine or TCDD, but did not contain detectable levels of the transcripts in the absence of an inducer (control tissues) (Fig. 1, #85, 88). Induction of CYP1A1 protein was not as pronounced as that of mRNA, based on the high constitutive levels of the protein in control tissues (compare Figs. 1 and 2a-b). The absence of CYP1A1 mRNA in some of the control and many of the non-explanted tissues could be due to enhanced degradation in the absence of inducing agents (Rumsby et al., 1996).

Among the accomplishments of the present study was our ability to detect CYP1A1 in whole tissue proteins. Hitherto, immunochemical studies have utilized microsomes. The use of whole homogenates was, thus, a novel way to analyze protein expression in animal tissues and allowed the detection of what may be CYP1A2 in most of the samples examined. Further studies will be necessary to confirm the identity of the putative lung CYP1A2 and its possible localization in subcellular fractions other than the endoplasmic reticulum.

Although only a small sample size has been examined, a pattern was observed that seemed to classify the induction as preferential for pyridine (Fig. 1, #85; Fig. 2a) or

TCDD (Fig. 1, #88; Fig. 2b). This observation suggests the involvement of different mechanisms in the induction of CYP1A1 by the two compounds. On the other hand, some specimens showed approximately equal levels of CYP1A1 in the control-, pyridine-, and TCDD-treated samples (Fig. 1, #104; Fig. 2c). It is interesting to note that these specimens were obtained from individuals who were ex-smokers or worked in polluted environments. The absence of induction in these cases suggests a maximum level of induction (perhaps by environmental chemicals), above which further induction is not possible (Rumsby et al., 1996). This study tested the effectiveness of pyridine as an inducer and used TCDD as a control for induction. The discovery that induction by pyridine was comparable to and often even greater than that by TCDD (Fig. 1, #104; Fig. 2a) was a surprising finding because TCDD is considered to be the prototypic and most potent inducer of CYP1A1.

Induction of CYP1A1 mRNA and protein was found to correlate positively with increased metabolism of B[a]P to reactive DNA-binding intermediates (Fig. 3). As the binding of epoxide forms of B[a]P is thought to be the initial step in carcinogenesis (Sims and Grover, 1974), the finding seems to indicate that tobacco smoke chemicals such as pyridine play an effective role in increasing lung cancer susceptibility.

Another surprising finding was the discovery of a significant number of CYP1A1 mutations in a local population sample. The polymorphisms observed in this study were those at the m2 and m4 loci, which have been shown to be most prevalent in Japanese and Slavic populations, respectively (Xu et al., 1996). Most of the five specimens genotyped seem to be heterozygous m2 or m4 mutants, but the restriction fragment length patterns of several samples were unclear (see Fig. 4, lanes 10-12). While the presence of a significant number of mutants in a group of lung cancer patients suggests a positive correlation between CYP1A1 polymorphism and lung cancer susceptibility, more samples will have to be examined to provide the statistical power necessary for assessing the relationship between genotype and inducibility of the enzyme.

In conclusion, the human lung explant culture system provides a suitable and more relevant model than peripheral blood lymphocytes for studies of the regulation of human pulmonary CYP1A1 expression.

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Table 1. Restriction Fragment Length Patterns of CYP1A1 Mutations[1]

CYP1A1		**Restriction**		**Fragment**
Variant	**Mutation**	**Enzyme**	**Genotype**	**Length (bp)**
m1	6235 T ▸ C		Wild type	899
			Mutation	693 ; 206
m2	4889 A ▸ G	*Msp*1 *BsrD*1	Wild type	149 ; 55
			Mutation	204
m3	5639 T ▸ C A	*Msp*1	Wild type	899
			Mutation	802 ; 97
m4	4887 C ▸	*Bsa*1	Wild type	139 ; 65
			Mutation	204

[1] adapted from Cascorbi et al. (1996)

Fig. 1. Agarose gel electrophoresis showing CYP1A1 mRNA levels in nonexplanted and explanted tissues cultured in the presence or absence of inducers. Con: control (DMSO); Pyr : pyridine; NE: non-explanted tissue; L: 100-bp DNA ladder.

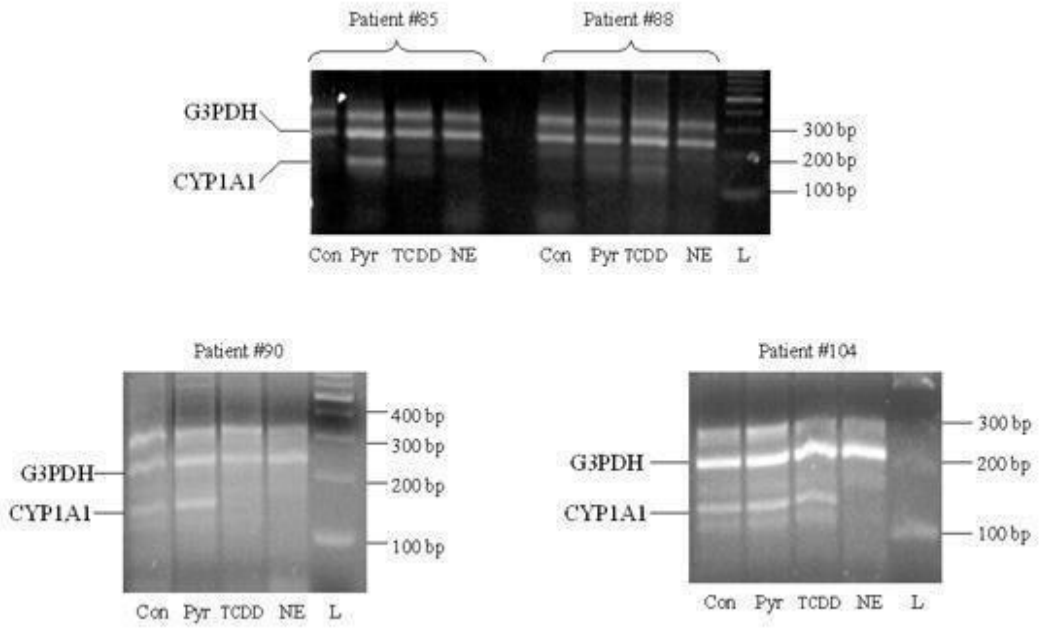


Fig. 2a. Western blot analysis of .25 £gg liver microsomal protein from snaphthoflavone-treated rats (BNF Lv) and proteins extracted from whole tissue homogenates from control (Con), pyridine-treated (Pyr),or TCDD-treated (TCDD) lung explants. Each lane represents 60 £gg total homogenate protein.
Note that pyridine is a better inducer than TCDD in this patient.

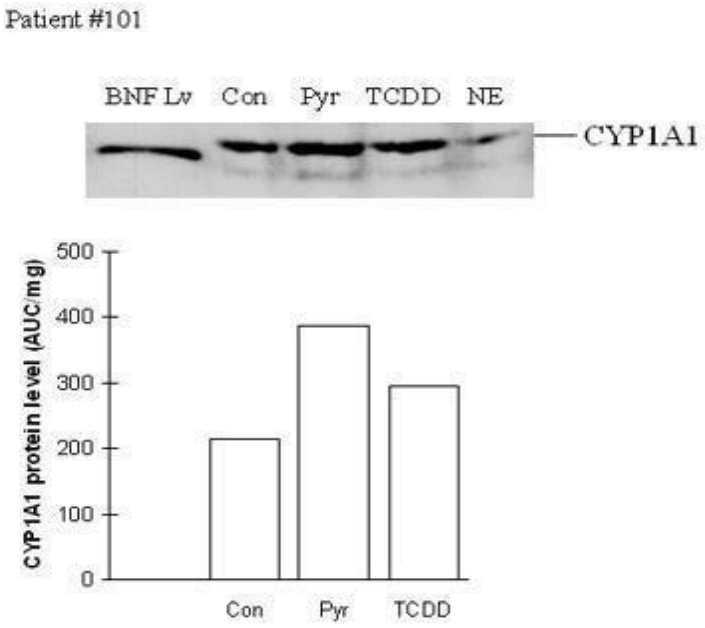
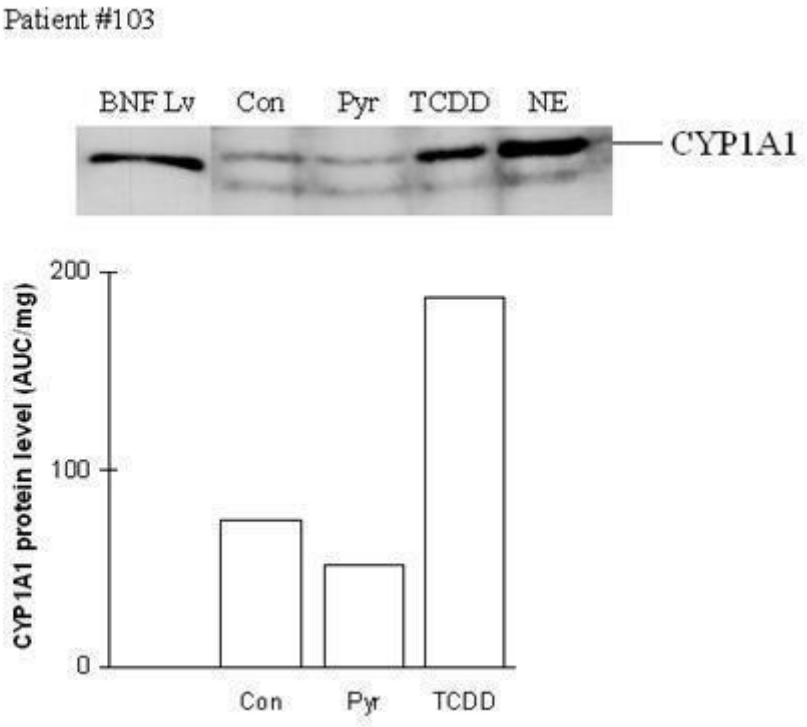


Fig. 2b. Western blot analysis of .25 £gg liver microsomal protein from snaphthoflavone-treated rats (BNF Lv) and proteins extracted from whole tissue homogenates from control (Con), pyridine-treated (Pyr), or TCDD-treated (TCDD) lung explants. Each lane represents 75 £gg total homogenate protein.



Note that

TCDD but not pyridine is an inducer in this patient.

Fig. 2c. Western blot analysis of .25 £gg liver microsomal protein from snaphthoflavone-treated rats (BNF Lv) and proteins extracted from whole tissue homogenates from control (Con), pyridine-treated (Pyr), or TCDD-treated (TCDD) lung explants. Each lane represents 100 £gg total homogenate protein.
Note the high levels of CYP1A1 in the control and the lack of induction by pyridine and TCDD in this patient.

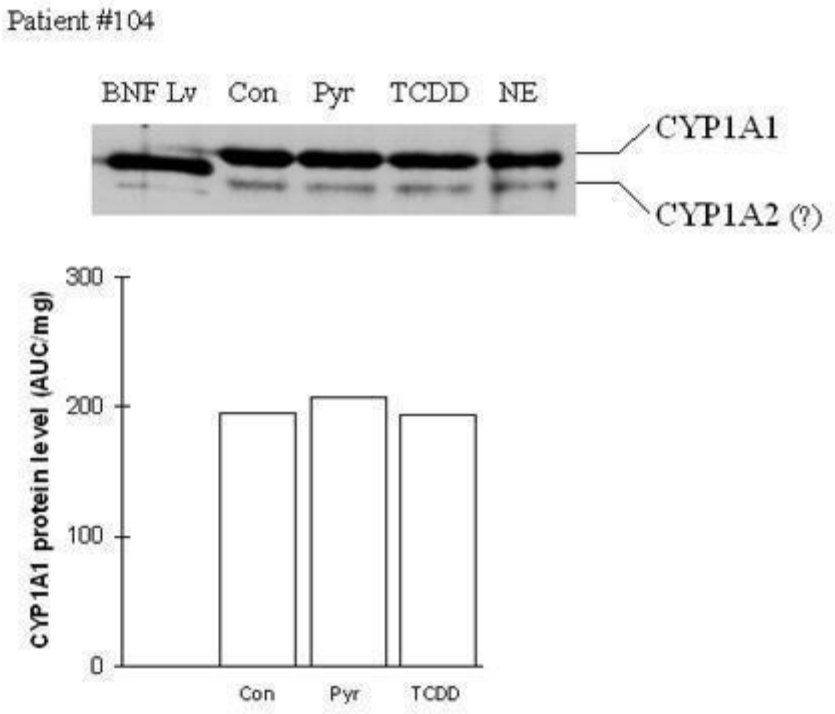


Fig. 3. The effects of TCDD and pyridine on the level of B[a]P adducts to the DNA of one patient. TCDD and pyridine induce CYP1A1-mediated bioactivation of B[a]P to reactive intermediates that covalently bind to endogenous pulmonary DNA.

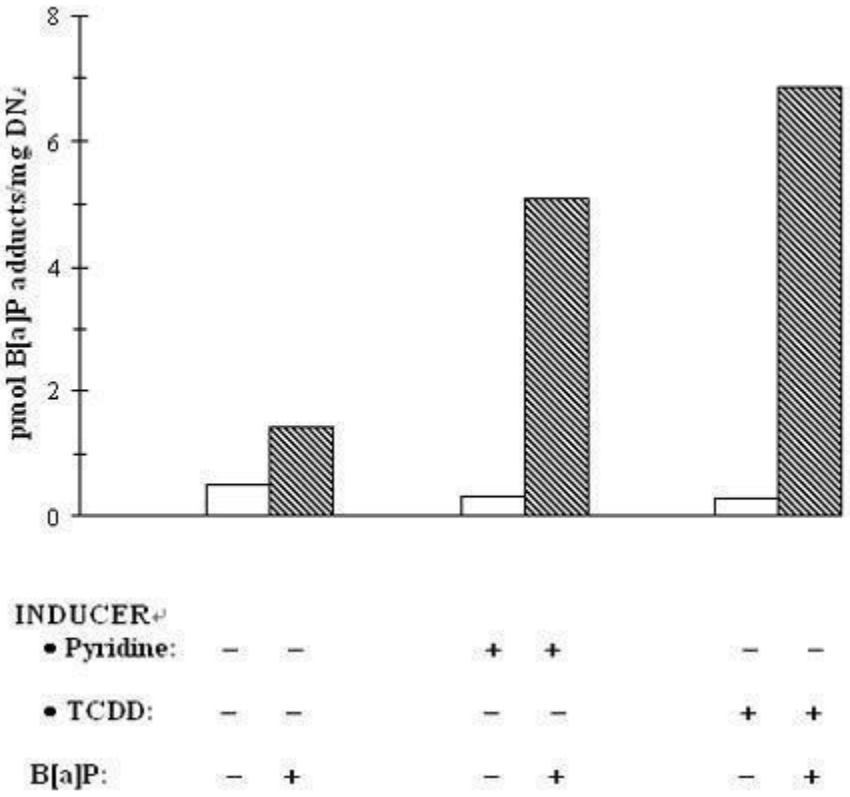


Fig. 4. Agarose gel electrophoresis of CYP1A1 DNA fragments after restriction enzyme digestion. Each lane represents a lung sample from an individual patient.

Lanes 1-5: Msp1 digestion testing m1 and m3 (all homozygous wild type) 6-10: BsrD1 digestion testing m2 (Lane 6: homozygous mutant; 7-10: heterozygous wild type/mutant)

11-15: Bsa1 digestion testing m4 (Lane 14: homozygous wild type (?); 11, 12, 15: heterozygous wild type/mutant; 13: homozygous mutant).