BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **241,** 79–85 (1997)
ARTICLE NO. RC977775

Evidence for CYP2D6 Expression in Human Lung

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Received October 27, 1997

The cytochrome P450 CYP2D6 gene (*CYP2D6*) expression was examined in samples from human bronchial mucosa and lung parenchyma using reverse transcription-polymerase chain reaction (RT-PCR) and immunochemistry. Except specimen from a patient previously genotyped as homozygous for a complete deletion of the gene, all tissue samples were positive. When compared to that in the liver, the mean level of *CYP2D6* mRNA was 3-fold lower in bronchial mucosa and 6-fold lower in lung parenchyma. To our knowledge, these data demonstrate for the first time the presence of CYP2D6 protein in human lung. They also indicate that the gene is nonuniformly distributed within this organ. The possibility that CYP2D6 has a role in lung carcinogenesis by locally activating inhaled chemicals should therefore be considered.

***Key Words:* *CYP2D6* expression; human lung; lung cancer; RT-PCR; immunochemistry.**

The lung represents a major target for cytotoxicity, necrosis and carcinogenesis since it is continuously exposed to inhaled environmental chemicals but also to all xenobiotics, through the general circulation [1-4]. Many of these xenobiotics are relatively inert substances that require metabolic activation for their cytotoxic and tumorigenic effects [1, 3, 5]. Metabolic activation pathways usually involved phase I enzymes through oxidation reduction reactions catalyzed by cytochrome P450 isozymes. The expression of some of them is subject to genetic polymorphism, resulting in large interindividual variations in enzyme activity, and probably responsible for susceptibility to some environmentally-based cancers [6-9].

The importance of these variations is exemplified by studies on the cytochrome P450 2D6 (CYP2D6) polymorphism for which four groups of individuals can be defined according to their phenotype: poor metabolizers (PM), intermediate metabolizers (IM), extensive metabolizers (EM) and ultrarapid metabolizers (UM) [10], which occur with varying prevalence in different populations [11]. The gene which codes for CYP2D6 (*CYP2D6*) has been localized to chromosome 22 where it forms part of the *CYP2D* gene cluster with two pseudogenes termed *CYP2D7P* and *CYP2D8P* [12]. The polymorphic CYP2D6 activity is caused by numerous allelic variants of the CYP2D6 gene [13, 14], as well as rearrangement of the *CYP2D* locus, including the deletion of the entire CYP2D6 gene [15].

Over the past decade, numerous epidemiological studies have examined the possible relationship between CYP2D6 phenotype and/or genotype and lung cancer with discrepant findings [for review see ref 16], suggesting or not an excess of EM among patients with lung cancer. If the CYP2D6 is associated with lung cancer, the mechanism remains unclear, especially as the enzyme has never been detected in the lung. In the present study, the expression of the CYP2D6 has been investigated in human lung parenchyma and human tracheobronchial mucosa, using both polymerase chain reactions after reverse transcription (RT-PCR) and immunoblotting studies.

MATERIALS AND METHODS

Samples

Prior to their examination for *CYP2D6* expression, each sample has been selected according to *CYP2D6* genotype previously determined using *Xba* I RFLP analysis for the detection of the entire *CYP2D6* deletion [15] and using SSCP analysis for the detection of point mutations [14]. One subject identified as homozygous for a deletion of the entire CYP2D6 gene was chosen as negative control for *CYP2D6* expression. All other patients selected had a normal CYP2D6 gene sequence.

Human lung parenchyma tissues used in this study were obtained from 7 patients undergoing surgery for lung carcinoma. Specimen were collected in macroscopically healthy areas adjacent to the tumor. Human tracheobronchial mucosae were obtained from biopsies of 7 patients undergoing bronchoscopy. Histologically normal human liver samples were obtained from 5 renal transplant donors, shortly

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0006-291X/97 $25.00

TABLE 1

Details of the Primers for Human *CYP2D6, CYP2D7P, CYP2D8P,* and *β-actin*

Gene	Primer	Sequence (5′ → 3′)	T°C[a]	$MgCl_2$ (mM)[b]	Size (bp)[c]
CYP2D6	Ex7f (sense)	GGAGATCGACGACGTGATAG	60	1	456
	Ex9R (antisense)	ACCAGGAAAGCAAAGACACC			
CYP2D7P/8P	Ex7f (sense)	GGAGATCGACGACGTGATAG	60	1	456
	Ex9RPS (antisense)	ACCAGAAAGCTGACGACACG			
β-Actin	ACT5F (sense)	GCACTCTTCCAGCCTTCC	58	1.5	228
	ACT3R (antisense)	GCGCTCAGGAGGAGCAAT			

[a] Optimized annealing temperature for each set of primers.
[b] Optimized $MgCl_2$ concentration for each PCR.
[c] Size of amplified fragments.

after circulatory arrest. All tissue samples were immediately frozen in liquid nitrogen and then stored at −80 °C until used.

RNA Isolation

Total RNA was extracted from frozen tissues using the RNA-PLUS kit according to manufacturer's instructions (Bioprobe Systems, Montreuil, France). Diethyl pyrocarbonate treated water was used in the final resuspension of RNA. The purity and the concentration of samples were determined by measuring the optical densities at 260 and 280 nm. Isolated RNA was then pretreated with RNase free DNase (DNase I, Amp grade, Gibco BRL, Gaithersburg, MD) to digest any contaminating genomic DNA.

First Strand cDNA Synthesis

One μg of RNA was combined with 0.5 μg oligo-dT primer, heated to 70 °C for 10 min and chilled on ice. The volume was then adjusted to 20 μl with a 50 mM Tris-HCl buffer, pH 8.3, containing 30 mM $MgCl_2$, 75 mM KCl, 10 mM DTT, 0.5 mM of each dNTP and 200 U of MMLV reverse transcriptase (SUPERSCRIPT II RNase H^- Reverse Transcriptase, Gibco BRL). The mixture was incubated at 42 °C for 1 h and then the reaction was stopped by heating at 70 °C for 15 min.

cDNA Amplifications

For amplification of synthesized CYP2D6 cDNAs, specific primers were designed based on the published nucleotide sequence of the gene and of its pseudogenes, *CYP2D7P* and *CYP2D8P*. Sense (Ex7f) and antisense (Ex9r) primers consisted of 20 bp complementary respectively to segments 3258-3277 and 4265-4246 in exons 7 and 9 of the CYP2D6 gene. Their sequence are listed in table 1. For PCR, 2 μl of the reverse transcriptase mixture were used. The sample volume was adjusted to 25 μl with a solution containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 μM of each primer and 0.625 U of Taq DNA polymerase (Boehringer Mannheim, Germany). The thermal profile used in a PTC150 thermocycler (MJ Research, Watertown, MA) included an initial denaturation step at 94 °C for 2 min, denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min 30 sec for a total of 36 cycles. A final extension step at 72 °C for 7 min was then carried out. Amplification of *CYP2D7P* and *CYP2D8P* were also performed with these conditions, using the same sense primer (Ex7f) and an antisense primer (Ex9rPS; Table 1) specific for these pseudogenes.

Amplification of human β-actin mRNA, was also performed as a constitutive reference indicator to control for differences in RNA degradation rates and purity in the different samples. The PCR was carried out under conditions described by Hamosh *et al.* [17].

To assess the kinetics of PCR amplification for each primer set, multiple reactions with increasing number of cycles have been performed using liver cDNAs. The rates of amplification were exponential between: 28 and 42 cycles for the β-actin primer set; 30 and 42 cycles for the *CYP2D6* primer set. All subsequent amplifications were carried out using the optimal cycle number for each primer couple: 35 cycles for the β-actin gene and 36 cycles for the CYP2D6 gene.

After amplification, a 10 μl aliquot of PCR products was analyzed by electrophoresis on a 1.5 % agarose gel containing ethidium bromide. For semi-quantitative RT-PCR analysis, the band intensities were measured by a CCD video camera (Bioprobe Systems) equipped with the Densylab™ software (Bioprobe Systems), allowing an assessment of CYP2D6 mRNA amount in studied samples. The level of CYP2D6 mRNA is presented as a percentage of β-actin mRNA.

To confirm the specificity of the amplification, the RT-PCR products were directly sequenced, after purification with "Qiaquick Spin" columns (Qiagen GmbH, Hilden, Germany), using the Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, ABI, Foster City, CA), as previously described [18]. The sequence of both strands was analyzed from at least two independent PCR amplifications.

Preparation of Microsomes

Human lung and liver microsomes were prepared as previously described [19]. Briefly, specimens were suspended in 50 mM Tris-HCl buffer, pH 7.4, containing 25 mM KCl, 250 mM saccharose, 5 mM β-mercaptoethanol, 5 mM magnesium acetate, and disintegrated with a glass-Teflon homogenizer. The mixture obtained was successively submitted to a 16,000 × g centrifugation for 20 min at 4 °C and a 180,000 × g ultracentrifugation for 1 h at 10 °C. The resulting pellet containing microsomal fractions was stored at −80 °C until used.

Immunological Studies

Sera. The rabbit polyclonal antibody raised against CYP2D6 was a generous gift from Pr. P. Beaune (Unité INSERM 75, Paris, France). It was obtained after immunization of a New Zealand rabbit with purified human CYP2D6 expressed in *Saccharomyces cerevisiae.* This serum was tested against different human CYP enzymes expressed in *Escherichia coli;* it did not recognize CYP1A2, CYP2C9, CYP2E1 and CYP3A4. The LKM-1 positive serum was obtained from a patient with Type II autoimmune hepatitis. Such a serum is known to recognize the CYP2D6 protein [20]. A rabbit preimmune serum and a serum from a healthy blood donor were used as negative controls.

Immunoblots. Proteins from 10 μg of human lung and liver microsomes were separated by electrophoresis in a 10-15 % gradient SDS-polyacrylamide gel. Blots were performed onto nitrocellulose (Hy-

bond-C extra, Amersham, Buckinghamshire, UK). Membranes were soaked for 2 h in TBS (0.01 M Tris/HCl buffer, pH 7.4, 0.15 M NaCl) containing 4 % defatted milk and 3 % Tween-20 before being incubated for 1 h at room temperature with the rabbit anti-CYP2D6 and the anti-LKM1 antibodies diluted 1 to 10,000 in TBS containing 1 % defatted milk. After three 15 min washes in TBS with 0.1 % Tween-20, membranes were incubated with 1 to 25,000 diluted secondary antibody conjugated to peroxidase (Sigma, St Louis, MO) for 1 h. After washing 3 times with TBS-Tween-20 and once with TBS, CYP2D6 epitopes were revealed using the enhanced chemioluminescence Western blotting detection system (ECL, Amersham).

RESULTS

CYP2D6 mRNA Expression

In a first step, the presence of the CYP2D6 mRNA has been examined in human lung using RT-PCR. For this purpose, RNA isolated from pulmonary parenchyma and tracheobronchial mucosa was reverse transcribed into cDNA and then amplified. The specificity of our PCR protocol was optimized in a series of reactions where parameters such as annealing temperature and magnesium concentration were varied (data not shown). A representative photograph of the RT-PCR amplification products in a UV-transilluminated agarose gel is illustrated in Fig. 1. The RT-PCR product generated with the *CYP2D6* primer set was a single band of 456 bp (Fig. 1A). This RT-PCR procedure consistently allowed the detection of fragments with the expected size in samples coming from both human lung parenchyma (Fig. 1A, lanes 1-3) and human tracheobronchial mucosa (Fig. 1A, lanes 5-7). As a negative control, a specimen corresponding to a subject who was homozygous for a deletion of the CYP2D6 gene was employed (Fig. 1A, lane 4). This sample was not amplified but generated, like all other samples, a 456 bp fragment when using a reverse primer (Ex9rPS, table 1) specific for *CYP2D* pseudogenes (Fig. 1B, lanes 1-7). The sequencing analysis of PCR fragments (data not shown) confirmed they originate from the target cDNA and ensured the specificity of the PCR reactions.

In a second step, the level of *CYP2D6* expression was evaluated in lung tissues, using β-actin as internal control, and compared to that in the liver. Fig. 2A and 2B illustrate ethidium bromide-stained agarose gels containing PCR products resulting from amplification of *CYP2D6* and *β-actin* cDNAs respectively. Interestingly, the CYP2D6 mRNA expression was different in lung tissue samples according to their histological type. The average CYP2D6 mRNA level in pulmonary parenchyma was 16 % (SEM = 3; n = 7) relative to actin mRNA and was 2-fold lower (32 %; SEM = 4; n = 6) than in bronchial mucosa (Fig. 2C). As expected, hepatic tissue exhibits the highest level of *CYP2D6* expression; the relative amount of CYP2D6 RNA (97 %; SEM = 14; n = 5) was 3-fold and 6-fold higher than in tracheobronchial mucosa and in lung parenchyma, respectively (Fig. 2C).

Immunoblotting Studies

Microsomal proteins from human lung and liver were analyzed by western immunoblot for reactivity with anti-LKM1 and rabbit anti-CYP2D6 antibodies (Fig. 3). These sera recognized in a cell lysate of CYP2D6 expressing *E. coli*, a band of about 50 kDa probably corresponding to the CYP2D6 protein (Fig. 3, lanes 1 and 6). In the microsomes of liver, lung parenchyma and bronchial mucosa, one major band co-migrating with this protein was recognized by both antibodies (Fig. 3 lanes 2-4 and 7-9). As expected, the staining was more intense in liver microsomes. By comparison between lung tissue samples, the signal appeared slightly stronger in tracheobronchial mucosa than in parenchyma (Fig. 3 lanes 3-4 and 8-9). Microsomes from the subject homozygous for the deletion of the CYP2D6 gene, showed no 50 kDa band (Fig. 3 lanes 5 and 10). Several faint bands with molecular masses lower than 50 kDa were visualized on immunoblots revealed by the rabbit anti-CYP2D6 serum (Fig. 3, lane 1-5). Such a staining was also observed when a preimmune serum was used (data not shown), suggesting that these bands were not specific.

DISCUSSION

The expression of the CYP2D6 gene in human lung was studied by RT-PCR and western immunoblot methods. The RT-PCR procedure that we developed allowed to distinguish, through the careful choice of primers, even closely related sequences, such as those of *CYP2D6, CYP2D7P* and *CYP2D8P*. The identification of the amplified fragments by sequencing analysis as well as the absence of amplification in a sample from a patient homozygous for the deletion of the CYP2D6 gene, ensured the specificity of our RT-PCR protocol. The result of our investigations argued in favour of CYP2D6 mRNA expression both in pulmonary parenchyma and in bronchial mucosa. In a recent study, CYP2D6 mRNAs have been also detected by RT-PCR in human lung tissue [21]. They were reported to be expressed as a series of alternatively splice forms and in particular, a variant skipping the exon 6. Nevertheless, in this study, the presence of the CYP2D6 protein has not been demonstrated.

Our immunoblotting studies showed the presence of a 50 kDa protein in all microsomal fractions of lung tissues, with reactivity for anti-CYP2D6 sera. These antibodies reacted with a cell lysate of CYP2D6 expressing *E. coli*, but did not react with microsomal fraction from a patient homozygous for the deletion of the CYP2D6 gene. All these findings gave evidence for the presence of the CYP2D6 protein in human lung, both in tracheobronchial mucosa and pulmonary parenchyma. As a consequence, CYP2D6 joints other P-450s already documented to be expressed in human respiratory tree,

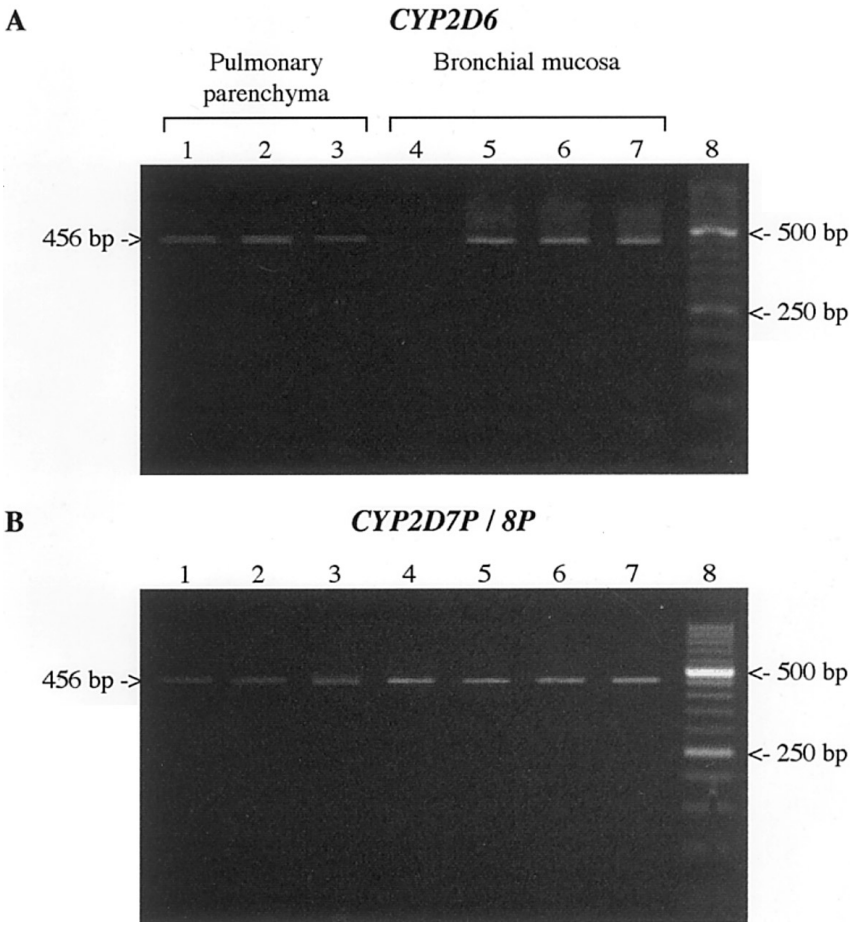

FIG. 1. Representative agarose gel electrophoresis of RT-PCR products obtained from the subsaturating amplification of (**A**) *CYP2D6* and (**B**) *CYP2D7P/8P* cDNAs in human pulmonary parenchyma (lanes 1-3) and bronchial mucosa (lanes 4-7). As expected RT-PCR yielded a single band near to 450 bp (predicted size 456 bp). Lanes 4 depict the results obtained for a subject who has been previously identified as homozygous for a deletion of the entire CYP2D6 gene. A 50 bp ladder is shown in the eighth lane of each gel.

including CYP1A1, 1B1, 2B1, 2B7, 2E1, 2F1, 3A4, 3A5 and 4B1 [22-24]. Using RT-PCR and immunochemistry Kivistö and colleagues [25] also investigated CYP2D6 expression in human lung. In contrast to our results, they found that both CYP2D6 mRNA and protein were not present at a detectable level in this tissue. Such discrepancies are difficult to explain but might be attributed to differences in technical approach.

By comparing the level of CYP2D6 mRNA expression in samples from distinctive portions of the lung, it was possible to distinguish a 2-fold lower CYP2D6 mRNA amount in the parenchyma with regard to the bronchial mucosa. These expression levels are not negligible even if they are much less important than that of liver which is known to be the major organ involved in CYP2D6 metabolism. In a recent study, the expression of phase I and phase II xenobiotic metabolism enzymes was evaluated in human bronchial epithelial cells and alveolar macrophages of non-smoking volunteers, using RT-PCR [23]. The authors also detected important intertissue differences in expression of such enzymes. Human lung has a particular complexity and is composed of more than 40 different cell types performing specific functions and located at distinctive portions of the organ. The difference in *CYP2D6* expression that we have observed between bronchial mucosa and parenchyma could be attributed to variation in constitutive level of expression or could result from variation in specialization of the cells constituting these tissues. However, our findings did not allow to deduce which respiratory cells express CYP2D6. Immunohistochemistry or a more sensitive analysis, based on a recently developed in situ RT-PCR method [26], should help us in characterizing *CYP2D6* expressing cell types in human lung and in understanding the specific susceptibility to xenobiotics of various lung compartments.

Environmental factors are clearly important in the pathogenesis of lung disease. The level of expression of enzymes, such as cytochromes P450, with the capacity to activate or to detoxify environmental chemicals may

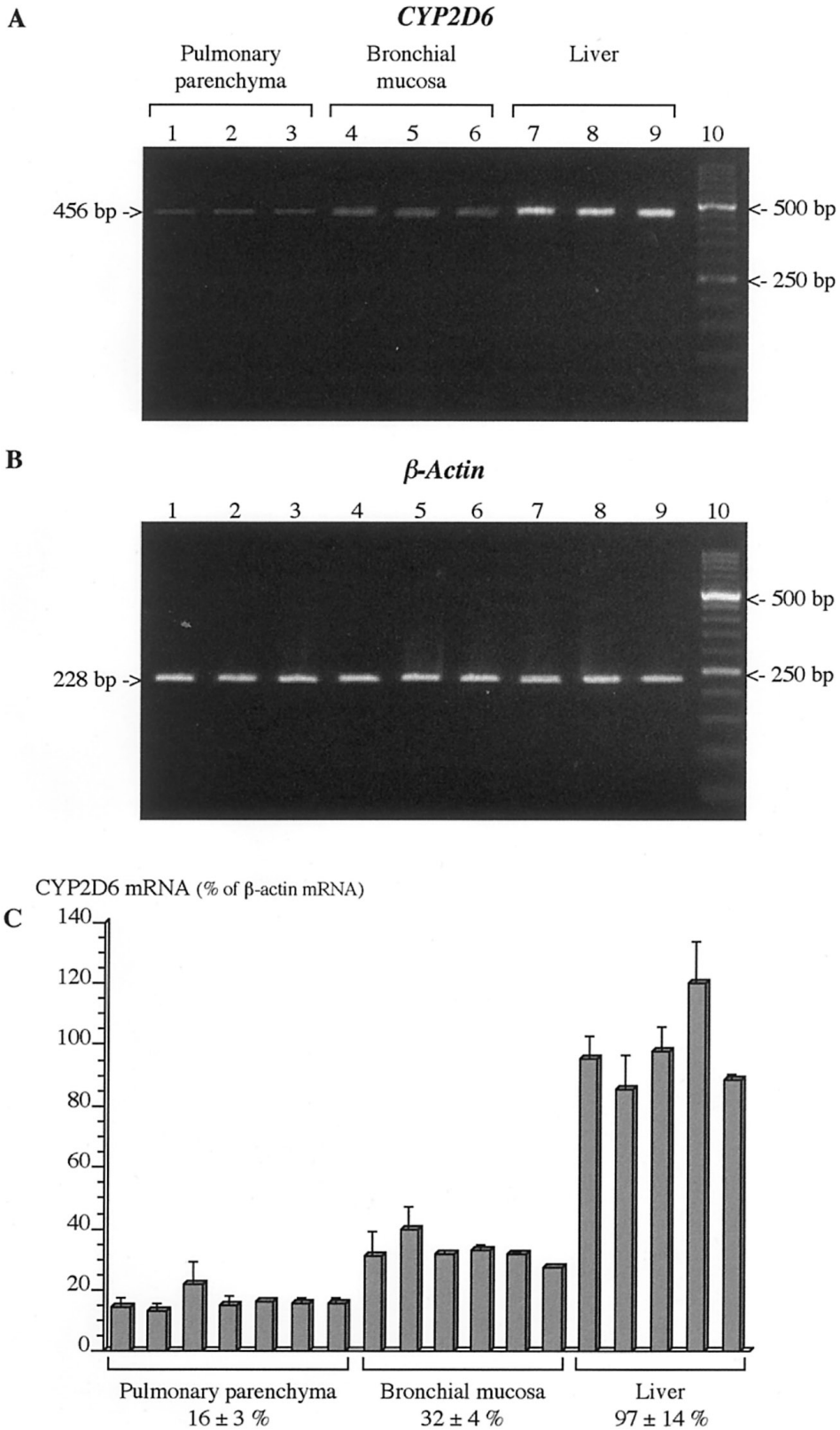

FIG. 2. Level of CYP2D6 mRNA in human pulmonary parenchyma (lanes 1-3), bronchial mucosa (lanes 4-6) and liver (lanes 7-9). (**A**) Representative agarose gel electrophoresis of fragments obtained from the amplification of CYP2D6 cDNA. (**B**) Agarose gel electrophoresis of RT-PCR products for β-actin. As expected, amplification yielded a single band between 200 bp and 250 bp (predicted size 228 bp). A 50 bp ladder is shown in the tenth lane of each gel. (**C**) Level of CYP2D6 mRNA in human pulmonary parenchyma, bronchial mucosa and liver. Results are presented as percentages of β-actin mRNA. The experiment to determine the abundance of CYP2D6 mRNA in each sample was repeated three times, and the error bars represent the level of reproducibility of our RT-PCR protocol. The amount of CYP2D6 mRNA was, on average, 16 % of actin mRNA for pulmonary parenchyma, 32 % for bronchial mucosa and 97 % for liver.

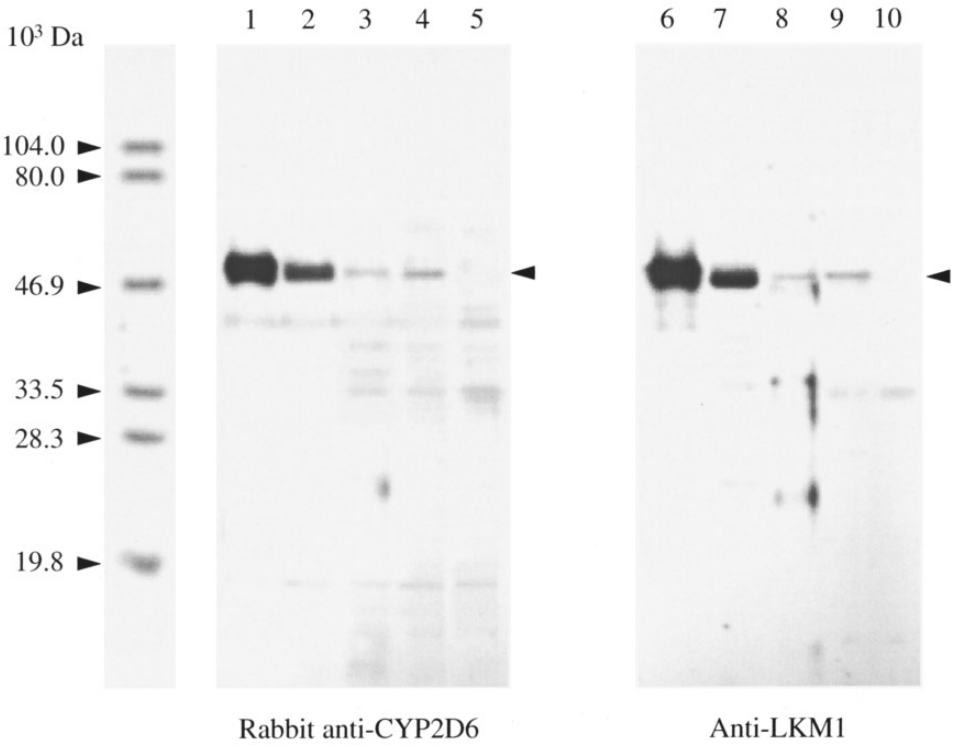

FIG. 3. Immunoblot analysis of the CYP2D6 protein. Lysates of CYP2D6 expressing *E. coli* were applied to the lanes 1 and 6, microsomes of human liver to the lanes 2 and 7, microsomes of human pulmonary parenchyma to the lanes 3 and 8, and microsomes of human bronchial mucosa to the lanes 4-5 and 9-10. Patterns 5 and 10 corresponded to samples of the subject homozygous for the deletion of the CYP2D6 gene. Each lane was loaded with 10 μg of protein, except for the lanes 1 and 6 (1 μg). Immunoblots were revealed with either rabbit anti-CYP2D6 antibodies (lanes 1-5) or LKM-1 positive serum (lanes 6-10), both diluted 1 to 10,000. Molecular mass markers are shown to the left of the figure.

therefore be an important determinant in homeostasis and pathology of human lung. CYP2D6, which is polymorphically expressed in the general population, is able to convert the procarcinogen 4(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a tobacco smoke-derived nitrosamine, to its active form [27]. A recent epidemiological study has suggested that smokers with both the highest CYP2D6 activity and daily tobacco consumption were at very high risk for lung cancer [28]. The role of tobacco smoke in the development of lung cancer is well known for squamous and small cell types, less so for adenocarcinoma, and not specifically assessed for large cell carcinoma [29]. It has been speculated that the strength of the association between smoking and lung cancer cell types could be related to cancer location, with more peripheral cancer types (such as adenocarcinoma and large cell carcinoma) showing weaker associations than more central tumors (such as squamous or small cell and oat cell carcinoma) [29]. An explanation might be based on the higher exposure to tobacco smoke particles to sites that are more central in the respiratory tract, but also on the potential higher procarcinogen activation via CYP2D6 metabolism in this lung area, as suggested by our findings that CYP2D6 is more expressed in bronchial mucosa than in pulmonary parenchyma.

In summary, we have demonstrated that CYP2D6 was significantly expressed in human lung and that this expression was higher in bronchial mucosa than in pulmonary parenchyma. The presence of CYP2D6 protein in the lung suggests that this enzyme may be of importance in the metabolism of inhaled xenobiotics including tobacco derived procarcinogens. All these findings provide some support that polymorphism of this enzyme might have a role in lung carcinogenesis.

ACKNOWLEDGMENTS

This study was supported by the Centre Hospitalier Régional et Universitaire de Lille, France, and by Rhône-Poulenc-Rorer through the Bioavenir programme.

REFERENCES

1. Baron, J., and Voigt, J. M. (1990) *Pharmac. Ther.* **47,** 419–445.
2. Roth, R. A., and Vinegar, A. (1990) *Pharmac. Ther.* **48,** 143–155.
3. Kikkawa, Y. (1992) *Lab. Invest.* **5,** 535–539.
4. Bond, J. A. (1993) *Pharmacol. Toxicol.* **72,** 36–47.

5. Daly, A. K., Cholerton, S., Armstrong, M., and Idle, J. (1994) *Environ. Health Perspect.* **102,** 55–61.
6. Wolf, C. R., Smith, C. A. D., and Forman, D. (1994) *Brit. Med. Bull.* **50,** 718–731.
7. Gonzalez, F. J. (1995) *Cancer. Res.* **6,** 193–201.
8. Raunio, H., Husgafvel-Pursianen, K., Anttila, S., Hietanen, E., Hiroven, A., and Pelkonen, O. (1995) *Gene* **159,** 113–121.
9. Smith, G., Stanley, L. A., Sim, E., Strange, R. C., and Wolf, C. R. (1995) *Cancer Surveys* **25,** 27–65.
10. Daly, A. K. (1995) *J. Mol. Med.* **73,** 539–553.
11. Bertilsson, L. (1995) *Clin. Pharmacokinet.* **29,** 192–209.
12. Kimura, S., Umeno, M., Skoda, R. C., Meyer, U. A., and Gonzalez F. J. (1989) *Am. J. Hum. Genet.* **45,** 889–904.
13. Daly, A. K., Brockmöller, J., Broly, F., Eichelbaum, M., Evans, W. E., Gonzalez, F. J., Huang, J.-D., Idle, J. R., Ingelman-Sundberg, M., Ishizaki, T., Jacqz-Aigrain, E., Meyer, U. A., Nebert, D. W., Steen, V. M., Wolf, C. R., and Zanger, U. M. (1996) *Pharmacogenetics* **6,** 193–201.
14. Marez, D., Legrand, M., Sabbagh, N., Lo Guidice, J.-M., Spire, C., Lafitte, J.-J., Meyer, U. A., and Broly, F. (1997) *Pharmacogenetics* **7,** 193–202.
15. Gaedigk, A., Blum, M., Gaedigk, R., Eichelbaum, M., and Meyer, U. A. (1991) *Am. J. Hum. Genet.* **48,** 943–950.
16. Caporaso, N., DeBaun, M. R., and Rothman, N. (1995) *Pharmacogenetics* **5,** S129–S134.
17. Hamosh, A., Trapnell, B. C., Zeitlin, P. L., Montrose-Rafizadeh, C., Rosenstein, B. J., Crystal, R. G., and Cutting, G. R. (1991) *J. Clin. Invest.* **88,** 1880–1885.
18. Marez, D., Sabbagh, N., Legrand, M., Lo Guidice, J.-M., Boone, P., and Broly, F. (1995) *Pharmacogenetics* **5,** 305–311.
19. Lo Guidice, J.-M., Périni, J.-M., Lafitte, J.-J., Ducourouble, M.-P., Roussel, P., and Lamblin, G. (1995) *J. Biol. Chem.* **270,** 27544–27550.
20. Manns, M. P., Griffin, K. J., Sullivan, K. F., and Johnson, E. F. (1991) *J. Clin. Invest.* **88,** 1370–1378.
21. Huang, Z., Fasco, M. J., Spivack, S., and Kaminski, L. S. (1997) *Cancer Res.* **57,** 2589–2592.
22. Czerwinski, M., McLemore, T. L., Gelboin, H. V., and Gonzalez, F. J. (1994) *Cancer Res.* **54,** 1085–1091.
23. Willey, J. C., Coy, E., Brolly, C., Utell, M. J., Frampton, M. W., Hammersley, J., Thilly, W. G., Olson, D., and Cairns, K. (1996) *Am. J. Respir. Cell Mol. Biol.* **14,** 262–271.
24. Anttila, S., Hukkanen, J., Hakkola, J., Stjernvall, T., Beaune, P., Edwards, R. J., Boobis, A. R., Pelkonen, O., and Raunio, H. (1997) *Am. J. Respir. Cell Mol. Biol.* **16,** 242–249.
25. Kivistö, K. T., Griese E.-U., Stüven, T., Fritz, P., Friedel, G., Kroemer, H. K., and Zanger, U. M. (1997) *Pharmacogenetics* **7,** 295–302.
26. Nuovo, G. J. (1994) *in* PCR *in Situ* Hybridization: Protocols and Applications, 2nd ed., Raven Press, New York.
27. Crespi, C. L., Penman, B. W., Gelboin, H. V., and Gonzalez, F. J. (1991) *Carcinogenesis* **12,** 1197–1201.
28. Bouchardy, C., Benhamou, S., and Dayer, P. (1996) *Cancer Res.* **56,** 251–253.
29. Morabia, A., and Wynder, E. L. (1991) *Cancer* **68,** 2074–2078.