Pergamon

European Journal of Cancer, Vol. 34, No. 6, pp. 914–920, 1998

Printed in Great Britain
0959-8049/98 $19.00+0.00

PII: S0959-8049(98)00034-3

Original Paper

Characterisation of Xenobiotic-metabolising Enzyme Expression in Human Bronchial Mucosa and Peripheral Lung Tissues

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The human respiratory epithelium is in direct contact with chemical carcinogens and toxins in inhaled air. Therefore, the activities of xenobiotic-metabolising enzymes in this epithelium could modulate respiratory toxicity and carcinogenesis. We determined the expression of several xenobiotic-metabolising enzymes, including phase I and phase II enzymes, in human bronchial mucosa and peripheral lung tissues. Reverse transcription–polymerase chain reaction (RT–PCR) analysis of phase I enzymes showed CYP1A1 and CYP2C (CYP2C8 and CYP2C18) mRNA expression in all of the 14 bronchial mucosa specimens. CYP2A6 and CYP2B6 mRNAs were found in 85% of the samples, whereas 50 and 90% of the tissues displayed CYP2E1 and CYP3A5 expression, respectively. However, CYP1A2, CYP2D6 and CYP3A4 mRNAs were not detected in all samples analysed. Normal human bronchial epithelial cells (NHBE cells) cultured in serum-free conditions showed reduced P450 expression in comparison with the bronchial mucosal samples. Similar to the bronchial mucosa, the peripheral lung tissues expressed CYP1A1, CYP2A6, CYP2B6, CYP2C (CYP2C8 and CYP2C18), CYP2E1 and CYP3A5 mRNAs, but did not show detectable levels of CYP2D6. Additional P450s, such as CYP1A2 and CYP3A4, were detected. The expression of CYP1A1, CYP1A2, CYP2B6, CYP2E1 and CYP3A4/5 in peripheral lung tissues was confirmed at the protein level, whereas CYP2A6 protein was undetectable. The use of specific primers for the detection of the phase II isoenzymes belonging to the glutathione S-transferase mu (GSTμ) and *N*-acetyl transferase (NAT) families showed that GSTM1 was expressed in 40% of the bronchial mucosa and 25% of the peripheral lung tissues, whereas GSTM3 and NAT1 mRNAs were found in all bronchial and lung samples. Finally, NAT2 expression was detected in all peripheral lung tissues, but was not detected in the bronchus. In conclusion, these results describing the diversity of the xenobiotic-metabolising enzymes expressed in the bronchus and lung tissues indicate that the human respiratory system could significantly and specifically contribute to the activation and metabolism of several environmental procarcinogens. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: cytochrome P450, phase I enzymes, phase II enzymes, gene expression, lung, carcinogens
Eur J Cancer, Vol. 34, No. 6, pp. 914–920, 1998

INTRODUCTION

THE PULMONARY system represents an early site of entry into the body for many toxicants and carcinogens, some of which require metabolic activation by cytochrome P450 (CYP)-dependent mono-oxygenases. Host factors play a role in individual susceptibility to cancer and variations in drug-metabolising enzymes in target tissues could be largely involved [1]. For example, a 75-fold interindividual variation in the metabolic activation of benzo(a)pyrene to its ultimate carcinogenic metabolite has been observed in human bronchial explants [2]. Our previous studies have demonstrated

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Received 17 Jul. 1997; revised 14 Nov. 1997; accepted 2 Dec. 1997.

that certain polycyclic aromatic hydrocarbons, *N*-nitrosamines and mycotoxins can be enzymatically activated to form DNA adducts in human bronchial [3] and lung [4] explants. Therefore, the characterisation of xenobiotic-metabolising enzymes and particularly of CYPs, in the human respiratory system is a crucial step for a better understanding of lung xenobiotic metabolism, lung toxicity and carcinogenesis. Nevertheless, the biochemical pharmacology of the human respiratory system has received relatively little attention in comparison with major target sites, as the level of CYP content is quite low in the lung relative to their hepatic counterparts. Recent studies have shown the expression of several CYPs in human lung tissues [5, 6]. As the majority of human lung cancers arise from the bronchial epithelium, it also appeared important to characterise better the xenobiotic-metabolising enzymes expressed at the site of tissue damage. Therefore, in this study, the expression of several phase I enzymes, including CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C (2C8, 2C9, 2C18 and 2C19), CYP2D6, CYP2E1, CYP3A4/7 and CYP3A5, was evaluated, by reverse transcription–polymerase chain reaction (RT–PCR) and Western blot analysis, in human bronchial mucosa, cultured bronchial epithelial cells and peripheral lung tissues. Finally, the expression of phase II enzymes, glutathione S-transferase mu (GSTμ; GSTM1, GSTM3) and *N*-acetyl transferases (NAT1, NAT2) was evaluated in both bronchial mucosa and lung tissues.

MATERIALS AND METHODS

Cells and culture conditions

Normal human bronchial epithelial cell (NHBE cells) obtained from explants of autopsy specimens and the SV40 T-antigen immortalised BEAS-2B cell lines [7] were cultured on fibronectin/collagen-coated flasks in serum-free medium, as previously described [8].

Human samples

Bronchial and peripheral lung tissues were obtained from post-mortem individuals who were cancer free. The details of the tissue donors summarised in Table 1. The bronchial tissue was dissected free of peripheral lung tissue and a sterile scalpel blade was used to scrape the mucosa which was immediately put in guanidinium thiocyanate solution [9] and frozen. For peripheral lung tissue, a sample approximately 3×3 cm was removed and immediately frozen in liquid nitrogen and stored at − 80°C until use.

RNA preparation

RNA extraction of the bronchial mucosa samples was carried out using the RNeasy total RNA purification system as described by the manufacturer (Qiagen AG, Basel, Switzerland). Between 10 and 70 μg of total RNA were obtained.

Approximately 100 mg of frozen peripheral lung tissues were minced in 2.5 ml guanidinium thiocyanate solution with a sterile scalpel blade and homogenised. RNA was extracted according to the method of Chomczynski and Sacchi [9]. Further RNA purification was carried out using the RNeasy total RNA purification system (Qiagen AG). Between 20 and 130 μg of total RNA were obtained.

cDNA synthesis and PCR reactions

Synthesis of single-stranded cDNA from 7.5 μg of total RNA was performed with the 'First strand cDNA synthesis kit' (Boehringer Mannheim, Rotkreuz, Switzerland) using oligo $d(T)_{15}$ as primer. The oligonucleotide primers used for PCR were synthesised by Mycrosynth (Windisch, Switzerland). Their sequence and PCR product length are described in Table 2. Amplification reactions were conducted in a final volume of 50 μl consisting of 2 μl cDNA, 20 mM Tris–HCl, pH 8.55, 16 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 150 μg/ml bovine serum albumin, 200 μM of each deoxynucleotide

Table 1. *Tissue donor history*

Patient number	Age (years)	Gender	Cause of death	Blood alcohol (%)	Cotinine (ng/ml)	Drugs	Comments
6043	61	F	MVA	0	0	Cocaine	
6174	20	M	GSW	0	0	None	
6177	52	M	Heart attack	0	0	None	Worked as fire fighter Took Procardin
6274	29	F	Seizure	0	2	Phenobarbital	Took Premarin
6279	30	M	GSW	0	0	None	
6287	30	M	Skull fracture	nd	0	nd	Chlorine/asbestos exposure, alchohol abuser, smoker (30 packs/year)
6296	53	M	MVA	0	0	None	
6311	65	F	GSW (face)	0	239	None	
6372	59	F	Haemorrhage	nd	nd	nd	
6433	29	M	MVA	0.22	246	Cocaine Psuedoepherine	
6452	22	M	MVA	nd	0	nd	Smoker (8 packs/year)
6530	32	F	Heart attack	0	1005	None	
6871	44	M	Heart attack	0	0	None	
6876	27	M	MVA	0.21	636	None	
6936	17	F	MVA	0	nd	None	
7291	16	M	GSW	0.02	nd	None	
11092	56	M	Head injury	nd	nd	nd	Took Vasotec
11093	36	M	MVA	0.24	327	nd	

F, female; M, male; GSW, gun shot wound; MVA, motor vehicle accident; nd, not done.

Table 2. Primer sequences

Gene	Forward primer	Reverse primer	PCR size
1A1	GGAGGCCTTCATCCTGGAGA	CCTCCCAGCGGGCAACGGTC	295 nt
1A2	GGAGGCCTTCATCCTGGAGA	TCTCCCACTTGGCCAGGACT	299 nt
2A6	CAACCAGCGCACGCTGGATC	CCAGCATAGGGTACACTTCG	423 nt
2B6	ACACAGTGAATTCAGCCACC	TGGTGTGTTGGGTGACAATG	289 nt
2C	CCAGAGGTCACAGCTAAAGT	CCTGCTGAGAAAGGCATGAA	344 nt
2C8	TTCATGCCTTTCTCAGCAGG	ATTTGTGCAGTGACCTGAAC	373 nt
2C9	TTCATGCCTTTCTCAGCAGG	TTGCACAGTGAAACATAGGA	403 nt
2C19	TTCATGCCTTTCTCAGCAGG	ACAGATAGTGAAATTTGGAC	277 nt
2C18	TTCATGCCTTTCTCAGCAGG	ATTTAGGAAGTCACCCAAGA	391 nt
2D6	CCTGCGCATAGTGGTGGCTG	GCTTCTCCCAGACGGCCTCA	353 nt
2E1	TGCCATCAAGGATAGGCAAG	AATGCTGCAAAATGGCACAC	356 nt
3A4/7*	TGACCCAAAGTACTGGACAG	CTATTCACAAAGTAATTTGAGG	385 nt
3A5	TGACCCAAAGTACTGGACAG	TGAAGAAGTCCTTGCGTGTC	239 nt
GSTM1	ACCATGGACAACCATATG	GCTCAAATATACGGTGGAGG	205 nt
GSTM3	GTAATGGATTTCCGCACA	GGTCAAATATACGGTTCTGA	204 nt
NAT1	TGGTGTCTCCAGGTCAATCA	TTCGGTATTTGCTGTCTTCT	364 nt
NAT2	TGGTGTCTCCAGGTCAATCA	TTTGGTGTTTCTTCTTTGGC	364 nt
Actin	GTTGCTATCCAGGCTGTG	CATAGTCCGCCTAGAAGC	738 nt

PCR, Polymerase chain reaction; nt, nucleotide. *The distinction between CYP3A4 and 3A7 can be evaluated by cutting the PCR product with HindIII. This restriction site is present only on the CYP3A4 PCR product.

triphosphate (Boehringer Mannheim), 50 pmol each of forward and reverse primer and 1.5 units of Taq DNA polymerase (Biotaq; Bioprobe Systems, Montreuil sous Bois, France). The reactions were heated for two cycles to 98°C for 1 min, 60°C for 2 min and 72°C for 2 min and then cycled 28 times through a 1 min denaturation step at 94°C, a 1 min annealing step at 60°C and a 2 min extension step at 72°C in a DNA thermal cycler apparatus (BioConcept, Allschwil, Switzerland). These conditions were used for all amplifications except for the NAT and CYP3A4/7 primers, where the annealing step was performed at 50°C. All reactions were conducted with actin primers as internal controls. RNAs from genetically engineered cell lines stably expressing different CYPs [10, 11] were used as positive controls for each RT–PCR experiment. Ten microlitres of the PCR products were separated on a 2% agarose gel and visualised by ethidium bromide staining.

Microsome preparation

The frozen tissues were minced with a sterile scalpel blade and homogenised in 25 mM Hepes buffer containing 1 mM EDTA, 100 mM NaOH, 1 mM dithiothreitol (DTT), 0.25 mM phenylmethylsulphonyl fluoride (PMSF) and 10% glycerin, pH 7.25. Microsomes were prepared from these homogenates by differential centrifugations [12], resuspended in 50 mM Tris–HCl pH 7.4, containing 150 mM NaCl, 1% Triton X-100, 1% Na-deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 0.25 mM PMSF and subjected to electrophoresis.

Western blot analysis

Peripheral lung microsome preparations (10 µg) and standard human CYP microsomal fractions (Gentest Corp, Woburn, Massachusetts, U.S.A.) were subjected to SDS–polyacrylamide gel electrophoresis. The separated proteins were transferred to nitrocellulose filters with a semidry electroblotter (Bioblock Scientific AG, Frenkendorf, Switzerland). The filters were incubated with specific antibodies against CYPs and developed with the ECL-Western blotting kit as described by Amersham (Rahn, Switzerland). The rabbit anti-rat CYP1A and CYP2B1 antibodies were kindly provided by F.J. Gonzalez (NCl, Bethesda, Maryland, U.S.A.). The rabbit anti-hamster CYP1A2 and CYP2E1 antibodies were a generous gift from M. Lasker (Veteran Administration Medical Center, New York, U.S.A.) and the rabbit anti-human CYP3A antibodies were kindly provided by S.A. Wrighton (Lilly Research Laboratories, Indianapolis, U.S.A.).

RESULTS

Evaluation of the specificity of CYP primers

In order to evaluate the specificity of the primers used for the amplification of CYP families with more than one isoenzyme, such as CYP1A, CYP2C and CYP3A, RNAs from genetically engineered cell lines expressing individual CYPs [13] were used as controls in RT–PCR. As shown in Figures 1 and 2, no cross-reactivity could be found between the CYP1A1 and CYP1A2 primers, the four different CYP2C and the CYP3A4 and CYP3A5 oligonucleotides. Moreover, the use of actin primers, as an internal control, in addition to the CYP-specific primers, did not alter the amplification process (Figure 1).

CYP expression in bronchial mucosa and epithelial cells

Fourteen samples of human bronchial mucosa were examined by RT–PCR for the expression of several phase I enzymes. The results shown in Figure 3 indicate that the bronchial tissues expressed detectable levels of CYP1A1, 2A6, 2B6, 2C, 2E1 and 3A5. Expression of CYP1A2, 2D6 and 3A4/7 could not be detected in any of the samples analysed. All bronchial mucosa samples expressed detectable CYP1A1 mRNA, although 5 displayed low levels of expression. CYP2A6 and 2B6 were found in 12 of 14 tissues analysed. Interestingly, the two samples (nos 6043 and 6177) which showed undetectable CYP2A6 did not express CYP2B6, suggesting that these two cytochromes appear to be co-expressed, as previously described in the liver [14]. All but

one tissue expressed CYP3A5 and low levels of CYP2E1 mRNA were detected in approximately 50% of the tissues. CYP2C expression was found in all samples analysed (Figure 3) and the use of specific primers for the four CYP2C members (Table 2, Figure 2) indicated that the bronchial tissues expressed CYP2C8 and CYP2C18, low levels of CYP2C9 but did not express CYP2C19 (Figure 4).

When the CYP expression in normal human bronchial epithelial cells (NHBE cells) at passages 1 and 2 was compared with the expression previously observed in the original bronchial mucosa (no. 6530), a general decrease in CYP levels was observed in the cultured cells (data not shown). Moreover, the SV40 T-antigen immortalised human bronchial epithelial cell line (BEAS-2B) [7], which has been previously described to retain the expression of most of the phase II enzymes [11], displayed limited CYP expression, since only CYP1A1 and low levels of CYP2B6 mRNAs could be detected (data not shown).

CYP expression in peripheral lung tissues: comparison with bronchial mucosa

Peripheral lung tissues were found to express detectable levels of CYP1A1 (4/6), CYP1A2 (4/6), CYP2A6 (2/6), CYP2B6 (6/6), CYP2C (4/6), CYP2E1 (4/6), CYP3A4 (4/6) and CYP3A5 (6/6) mRNAs (Table 3). Among the CYPs evaluated in this study, only CYP2D6 expression could not be detected in any of the samples. A differential pattern of CYP expression could be observed between peripheral lung tissues and the bronchial mucosa, suggesting tissue-specific regulation (Tables 2 and 3). CYP3A4 and CYP1A2 mRNAs were observed in approximately 70% of peripheral lung tissues analysed, but were never detected in the bronchial mucosa. Interestingly, some discrepancies also exist in CYP expression between lung and bronchus from the same individual (Table 3). In a few cases, the CYPs normally found in both tissues, i.e. CYP1A1, CYP2A6 and CYP2E1, were better or only expressed in either the bronchial mucosa or the peripheral lung tissue. For instance, CYP1A1 expression could be detected only in the bronchial mucosa sample no. 6452, whereas this isoenzyme was found only in the peripheral lung tissue from sample no. 6936. Further analysis of CYP2C expression indicated that CYP2C8 and CYP2C18 were the main members expressed in the lung tissues, as previously found for the bronchial mucosa (Figure 4).

In order to evaluate whether the CYP RNA levels found in the lung tissues correspond to detectable protein expression, Western blot analyses were performed. As shown in Figure 5, CYP1A1, CYP1A2, CYP2E1, CYP3A and low levels of CYP2B6 proteins were expressed in microsomal fractions of different lung samples. The levels of protein expression generally correlated with the mRNA levels, except for sample no. 6452 which was CYP1A1 negative by RT–PCR, but showed detectable levels of CYP1A1 protein (Table 3, Figure 5). Moreover, several attempts to detect CYP2A6 expression were all unsuccessful, probably due to the low RNA expression in the lung samples. In the bronchial mucosa, CYP1A1 expression was also detected at the protein level (data not shown), nevertheless the limited amount of epithelial tissue did not allow further protein analysis.

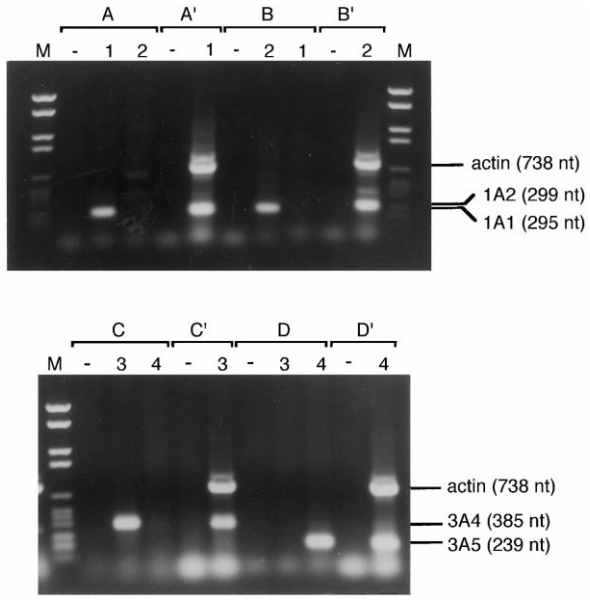

Figure 1. Specificity of the CYP1A1/1A2 and CYP3A4/3A5 primers. Total RNAs from genetically engineered human bronchial cell lines were reverse-transcribed and amplified by polymerase chain reaction as described in Materials and Methods. Lanes 1, 2, 3 and 4, CYP1A1-, CYP1A2-, CYP3A4- and CYP3A5-expressing cells, respectively; −, negative control; M, DNA marker VI from Boerhinger. A, CYP1A1 primers; A′, CYP1A1 + actin primers; B, CYP1A2 primers; B′, CYP1A2 + actin primers; C, CYP3A4 primers; C′, CYP3A4 + actin primers; D, CYP3A5 primers; D′, CYP3A5 + actin primers.

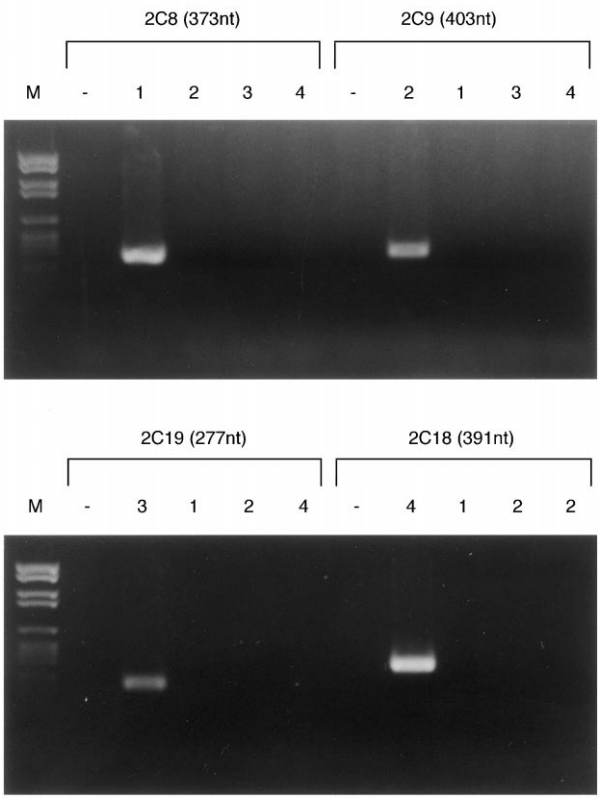

Figure 2. Specificity of the CYP2C8, CYP2C9, CYP2C18 and CYP2C19 primers. cDNA from four different CYP2C-expressing cells was amplified with CYP2C8, CYP2C9, CYP2C18 and CYP2C19 primers, as designated on the top of each gel. In parentheses is the expected polymerase chain reaction fragment length. Lanes 1, 2, 3 and 4, CYP2C8-, CYP2C9-, CYP2C18- and CYP2C19-expressing cells, respectively; −, negative control; M, DNA marker.

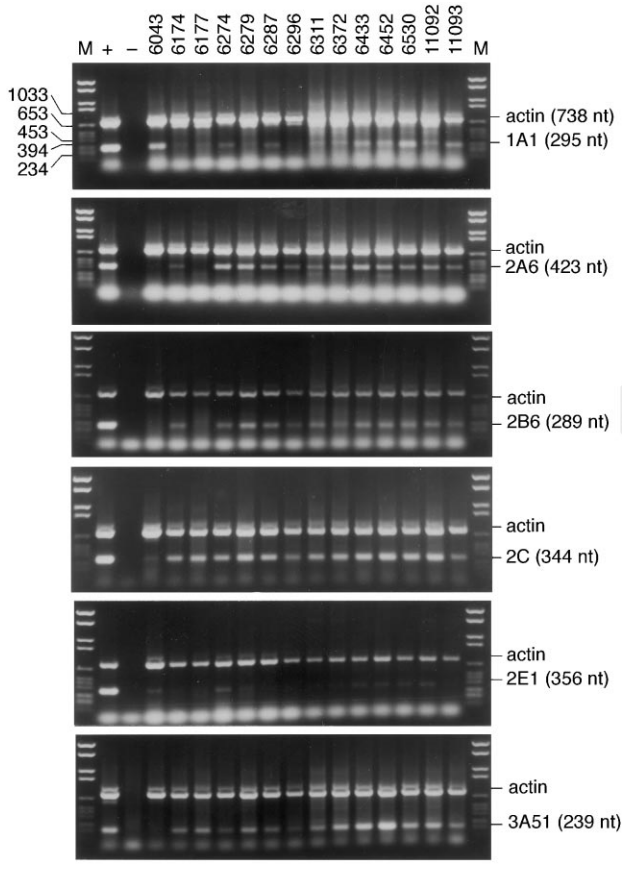

Figure 3. Cytochrome P450 (CYP) mRNA expression in human bronchial mucosa. Total RNAs from 14 human bronchial mucosa were reverse-transcribed and amplified by polymerase chain reaction using CYP1A1, CYP2A6, CYP2B6, CYP2C, CYP3A4 and CYP3A5 (top to bottom) in the presence of actin primers. +, CYP-expressing cells as positive controls; –, negative controls; M, molecular marker.

Phase II expression in bronchial mucosa and peripheral lung tissues

The analysis of GSTμ and NAT expression by RT–PCR indicated that all the bronchial mucosa and peripheral lung tissues tested showed detectable levels of GSTM3 and NAT2 mRNAs (Table 4). In addition, all the peripheral lung tissues tested expressed NAT2, whereas none of the bronchial mucosa were found to be positive. Finally, GSTM1 mRNAs were detected in 42% and only 25% of the bronchial mucosa and peripheral tissues, respectively.

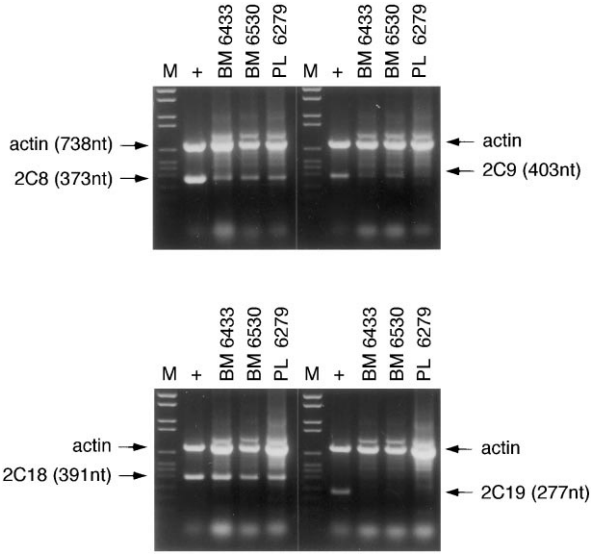

Figure 4. CYP2C isoenzymes expression in bronchial mucosa and peripheral lung tissues. Total RNAs from bronchial mucosa (BM) and peripheral lung tissues (PL) were reverse-transcribed and amplified by polymerase chain reaction using CYP2C8-, CYP2C9-, CYP2C18- and CYP2C19-specific primers in the presence of actin primers. +, CYP-expressing cells as positive controls; M, molecular marker.

DISCUSSION

We investigated the expression of 12 different CYP isoenzymes with a panel of primers in both human bronchial and lung tissues and showed that the pulmonary system is a potential active site for metabolising environmental procarcinogens. RT–PCR is a very powerful technology which allows detection of low gene expression in material with limited availability. However, the choice of the oligonucleotide primers is critical to avoid cross-reactivity, particularly in the detection of superfamily genes with numerous sequence homologies. For this purpose, we evaluated the specificity of our primers, by using RNAs from recombinant CYP-expressing cells [13], and showed no detectable cross-reactivity.

Previous studies using immunochemistry, Western blot analysis or catalytic activities have reported the detection of CYP1A protein in human lungs [15, 16]. Because of the limited specificity of the antibodies, as well as of the enzymatic activities, the exact isoenzymes expressed were not always clearly defined. Interestingly, we detected both CYP1A1 and CYP1A2 mRNAs in the peripheral lung tissues. The presence

Table 3. Cytochrome P450 (CYP) mRNA expression in bronchial mucosa and peripheral lung tissues

	No. 6177		No. 6452		No. 6871		No. 6876		No. 6936		No. 7291	
	BM	PL	BM	PL	BM	PL	BM	PL	BM	PL	BN	PL
CYP1A1	+	+	+	–	–	–	nd	+	–	+	+	+
CYP1A2	–	+	–	+	–	–	nd	–	–	+	–	+
CYP2A6	–	–	+	–	+	–	nd	–	+	+	+	+
CYP2B6	–	+	+	+	+	+	nd	+	+	+	+	+
CYP2C	+	–	+	–	+	+	nd	+	+	+	+	+
CYP2D6	–	–	–	–	–	–	nd	–	–	–	–	–
CYP2E1	–	–	+	–	–	+	nd	+	–	+	+	+
CYP3A4	–	–	–	–	–	+	nd	+	–	+	–	+
CYP3A5	+	+	+	+	+	+	nd	+	+	+	+	+

BM, bronchial mucosa; PL, peripheral lung tissue; –, undetectable; nd, not done.

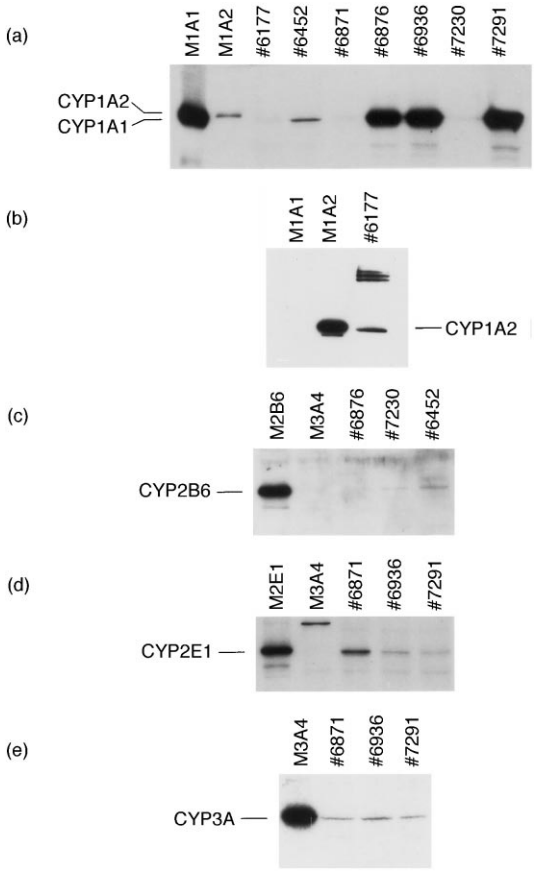

Figure 5. Cytochrome P450 (CYP) protein expression in lung microsomes. Ten micrograms of standard human CYP1A1 (M1A1), CYP1A2 (M1A2), CYP2B6 (M2B6), CYP3A4 (M3A4) or CYP2E1 (M2E1) microsomal fraction and peripheral lung microsomes (#) were subjected to electrophoresis. The blots were developed with anti-rat CYP1A (a), anti-hamster CYP1A2 (b), anti-rat CYP2B1 (c), anti-hamster CYP2E1 (d) and anti-human CYP3A (e).

of CYP1A2 mRNAs in lung was quite surprising, since this CYP has previously been considered as specific for hepatic tissues [17]. This result was further strengthened by the fact that no cross amplification of CYP1A1 was observed with the CYP1A2 primers and that the amplified DNA band was successfully cut at the Ava II restriction site, which is normally present in the CYP1A2 but not in the CYP1A1 PCR product (data not shown). Finally, the expression of the *CYP1A2* gene in the lung tissues was further confirmed at the protein level by using specific antibodies. Interestingly, the individual from whom the lung sample (no. 6177) showing a high level of CYP1A2 protein expression was obtained, had worked as a fire fighter. Over 40 cell types are required to perform the diverse functions of the respiratory tract, including 17 types of epithelium [18]. The CYP1A protein has been mainly localised in the type II alveolar epithelium [19]. We showed that CYP1A2 expression is restricted to lung peripheral tissues, as no detectable levels of CYP1A2 could be found in the bronchial epithelium. However, vascular endothelial and interstitial cells represent over 60% of the number of cells in the adult human lungs [18] and could be the source of CYP1A2 expression. This hypothesis is supported by a recent report describing CYP1A2 expression in human endothelial cells [20]. No evident correlations between cotinine blood levels (Table 1) and CYP1A1 expression could be found. Interestingly, the two tissue donors with detectable cocaine in their blood displayed high levels of CYP1A1 mRNA expression.

Among the studies examining CYP3A expression in human lungs, conflicting results have been obtained [6, 16, 21]. In the present paper, we clearly demonstrated that only CYP3A5 mRNAs were expressed in bronchial mucosa, whereas both CYP3A4 and CYP3A5 mRNAs could be detected in peripheral lung tissues. Interestingly, Anttila and colleagues have recently demonstrated that CYP3A5 is the predominant CYP3A protein expressed in human lungs [22].

The presence of CYP proteins belonging to the CYP2C subfamily in human lung microsomes has been recently reported [6]. Here, we determined that CYP2C8 and CYP2C18 were expressed both in bronchus and in peripheral lung tissues, whereas low levels of CYP2C9 could also be detected in bronchial mucosa. Although the human CYP2C subfamily appears to metabolise principally a number of clinically used drugs, chemical carcinogens, including nitrosamines, have been reported to be metabolically activated by the CYP2C enzymes [23].

Finally, our present data confirm the expression of other CYP enzymes, including CYP2A6 and CYP2E1 [6], as well as the CYP2B6 protein previously described at the RNA level in the human respiratory tract [24].

Variations in susceptibility to human lung cancer have been associated with the genetic polymorphisms in the expression of several xenobiotic-metabolising enzymes [1]. Among them, the relationship between lung cancer risk, tobacco smoke and the CYP2D6 phenotype has been extensively studied, but the association is controversial [25]. Recently, it was clearly demonstrated that high CYP2D6 activity is a risk factor only among heavy smokers [26]. This finding could be explained by the fact that there is no detectable CYP2D6 expression in human bronchus and lungs, even in tissues from patients with a high level of blood cotinine (Table 1). The activation by CYP2D6 of procarcinogens, such as the *N*-nitrosamine 4-(methylnitrosamino)-1(3-pyridyl)-1-butanone (NNK), most likely occurs in the liver from where reactive intermediates could be transported to the lungs via the circulation system, when the detoxification

Table 4. *Glutathione S-transferase and* N*-acetyl transferase expression in bronchial mucosa and peripheral lung tissues*

	% of positive tissues*			
	GSTM1 (*n*)	GSTM3 (*n*)	NAT1 (*n*)	NAT2 (*n*)
Bronchial mucosa	42 (12)	100 (14)	100 (4)	0 (4)
Peripheral lung	25 (4)	100 (5)	100 (7)	100 (5)

*The values represent the % of positive tissues as determined by reverse transcription–polymerase chain reaction (RT–PCR).
n, number of samples analysed.

pathway is overloaded. Therefore, we speculate that high levels of some procarcinogens are probably necessary to produce sufficient amounts of reactive intermediates able to induce genotoxic effects in the lungs.

GSTs are a multigene family of enzymes which play a central role in the detoxification of carcinogens by conjugating the xenobiotics with glutathione. Among the GSTμ family, the GSTM3 isoform was detected in 100% of the samples, whereas the polymorphic GSTM1 was found to be expressed in approximately 40% of the bronchial tissues. This result is in agreement with the finding that, in most racial groups, 40–50% of subjects are homozygous for the null allele and lack GSTM1 [27]. The lower frequency of GSTM1 expression (25%) found in lungs was probably due to the small number of samples analysed ($n = 4$). Genetic polymorphism has also been described for the *NAT* genes involved in the activation and detoxification of arylamine carcinogens. NAT2, was detected in all lung samples tested, but not in the bronchial mucosa, suggesting tissue-specific regulation. In contrast, the NAT1 isoform was expressed in all bronchial and lung samples.

In conclusion, this study clearly shows that the pulmonary system expresses, with interindividual variation, numerous phase I enzymes which catalyse the activation of environmental procarcinogens. Although, the metabolic capacity of the pulmonary system is low in comparison with the liver, the location and the function of this organ suggest an important role in the aetiology of chemical-associated cancers.

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Acknowledgements—The authors gratefully acknowledge Dr E.A. Offord for critical reading of the manuscript. This work was supported in part by funds provided by CRADA 0123, Nestlé Research Centre, Nestec Ltd and by the Swiss Federal Office of Education and Science, in relation to EU project AIR2-CT93-0860.