Virchows Archiv (1995) 426:243–247

ORIGINAL ARTICLE

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Immunohistochemical localization of cytochrome P450 2E1 in human pulmonary carcinoma and normal bronchial tissue

Received: 26 October 1994 / Accepted: 20 December 1994

Abstract Cytochrome P450 2E1 (CYP2E1) is a major xenobiotic-metabolizing enzyme but data concerning its extrahepatic expression are few. CYP2E1 can metabolically activate many procarcinogens and therefore its presence in the lung might play a role in bioactivation of procarcinogens, so we studied the expression and localization of CYP2E1 in primary pulmonary carcinomas and surrounding normal bronchial tissue from 28 patients. Seromucous glands showed expression of CYP2E1 in 19 and bronchial epithelium in 18 of the 28 samples of normal bronchial tissue. Thirteen of the corresponding cases of primary pulmonary carcinoma showed staining for CYP2E1. In 11 of these 13 cases, CYP2E1 was also present in normal bronchial tissue. There was no statistically significant difference in the expression of CYP2E1 between adenocarcinomas and squamous cell carcinomas. No association was observed between the expression of CYP2E1 in tumour tissue and normal bronchial tissue. However, there was a significant correlation between the expression of CYP2E1 in seromucous glands and bronchial epithelium (r=0.61, P<0.01) of normal tissue. We conclude that CYP2E1 can be present in both normal and neoplastic bronchial tissue.

Key words CYP2E1 · Immunohistochemistry · Pulmonary carcinoma

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Introduction

The cytochrome P450 (CYP) enzymes play a major role in the oxidative metabolism of drugs and other xenobiotics. Although CYP enzymes are primarily expressed in the liver, they are also found in extrahepatic organs such as the lung. The total CYP content of the lung is low compared with the liver, but considering the high rate of blood flow through the lungs and their place in the circulation, the lung may play a pivotal role in extrahepatic drug metabolism.

P450 monooxygenase activities in animal lungs have been well characterized and reside in Clara cells (nonciliated bronchiolar epithelial cells) and type II pneumocytes (alveolar type II cells) [1, 10, 16]. In man, many drugs are known to be taken up by the lung, but much less is known about drug metabolism and localization of CYP enzymes in this organ [13]. CYP enzymes so far identified in human lung tissue include CYP1A1, CYP2B7, CYP2E1, CYP3A and CYP4B1 [3, 11, 14, 17].

Differences in the expression of drug metabolizing enzymes between normal and cancerous lung tissue have been described. For example, Cohen et al. [2] reported a major difference in the routes of conjugation between normal lung tissue and tumour tissue from patients with lung cancer, and McLemore et al. [14] showed evidence for altered regulation of the CYP1A1 gene in primary pulmonary carcinomas. A recent immunohistochemical study from our laboratory found no correlation between CYP3A expression in tumour tissue and normal bronchial tissue [11].

CYP2E1 is a major xenobiotic-metabolizing enzyme: examples of the numerous substrates that have been identified for human CYP2E1 include ethanol, acetaminophen, enflurane, halothane, acetone and carbon tetrachloride [12]. Among the substrates are also many potentially important chemical carcinogens like benzene, dimethylnitrosoamine and vinyl chloride [6]. Since CYP2E1 is capable of metabolically activating many procarcinogens, the presence of CYP2E1 in the lung

may play an important role in the bioactivation of some inhaled procarcinogens.

Wheeler and coworkers [17] have previously detected CYP2E1 in human lung microsomes using two different methods, a radioimmunoassay and western blotting. However, there are no data on the localization of CYP2E1 in specific cell types of human lung tissue. The aim of the present work was to study, by means of immunohistochemistry, expression and localization of CYP2E1 in primary lung tumours and normal lung tissue from the same individuals.

Materials and methods

The material for this study consisted of paraffin wax blocks of specimens of both normal and cancerous lung tissue, retrieved from the files of the Department of Pathology of Robert-Bosch-Krankenhaus. Twenty-eight patients (23 males and 5 females) who had undergone surgery for primary pulmonary carcinoma were included. There were 13 squamous cell carcinomas, 8 adenocarcinomas, 4 adenocarcinomas with squamous differentiation, 2 large cell carcinomas and 1 small cell carcinoma. The age of the patients ranged from 41 to 75 years (mean, 58 years). Twenty patients were smokers. Detailed histories of alcohol use were not available, but most of the patients either did not report any use of alcohol (14 patients) or were considered moderate drinkers (consumption ≤40 g of ethanol/day; 10 patients). Three patients were excessive drinkers (alcoholics) and in one case the drinking history was unknown. Following operation, the removed lung or lobe was fixed in formalin within 12–48 h. In each case, a sample was obtained from both the tumour and surrounding normal bronchial tissue. In addition, a sample of peripheral, normal lung tissue was available from 11 patients. From each sample, 3 μm thick paraffin sections were prepared by standard methods.

A rabbit polyclonal antibody raised against human CYP2E1 was used for immunostaining. This antibody was obtained after immunization of a New Zealand rabbit with the amino acids 145–463 of the human CYP2E1 protein, expressed in *Escherichia coli* after cloning of the corresponding cDNA in the pET 23c vector. The serum was used at a dilution of 1:1000 in western blots and it was specific for the CYP2E1 isoform. The serum was tested against different human CYP enzymes expressed in yeast; it did not recognize CYP1A1, CYP1A2, CYP2C8, CYP2C9, CYP2C18, CYP2D6, CYP3A4 or CYP3A5. Immunostaining was performed with a modification of the avidin-biotin-peroxidase complex (ABC) technique [8]. The optimal dilution of the first antibody was determined to be 1:200. The second, biotinylated antibody was goat anti-rabbit, diluted 1:200. The visualization of the ABC complex was achieved with hydrogen peroxide diaminobenzidine. This method has been described earlier [4] and was recently applied in this laboratory to study the expression of CYP3A in liver tumours [5] and in the lung [11], and the expression of glutathione S-transferase in bronchial tissue [18].

To control the specificity of the immunostaining procedure, the following experiments were carried out: (1) omitting the primary antibody; (2) omitting the secondary antibody; (3) omitting the ABC complex; (4) omitting (1) and (2); (5) omitting (1), (2) and (3); (6) nonsense controls with antibodies raised against the Epstein-Barr virus and the hepatitis B core antigen; (7) a positive control (liver).

In an additional experiment, immunostaining of the tumour samples was performed as described above, except that the staining was intensified by adding nickel acetate and cobalt citrate to the incubation solution [7].

The staining intensity of both tumour cells and normal bronchial tissue was estimated as follows: 0, none; 1, slight; 2, moderate; 3, strong. The estimated percentages of positive tumour cells were classified as follows: 0, none; 1, <10%; 2, 10–50%; 3, >50%. The same classification was used for evaluation of the seromucous glands and bronchial epithelium in samples of normal bronchial tissue. The product of the scores for staining intensity and extent of staining was defined as the immunoreactivity score (IRS), which could thus have values 0–4, 6, or 9. To avoid possible false positive results, however, only IRS values ≥3 were considered positive. Spearman's rank correlation coefficients (r) were calculated to study the association between the IRS values of different tissues (the comparisons made are detailed in the Results). The Mann-Whitney test was used to compare the expression of CYP2E1 in adenocarcinomas and squamous cell carcinomas and to study the association between reported consumption of alcohol and CYP2E1 expression in normal bronchial tissue (represented by seromucous glands).

Results

The distribution of CYP2E1 in normal bronchial tissue is outlined in Table 1. In the tumour cells we observed homogeneous immunostaining for CYP2E1 in the cytoplasm. The nuclei and the cytoplasmic membrane were always unstained. In the squamous cell carcinomas, immunostaining was stronger in more differentiated areas, while no regular staining pattern was apparent in the adenocarcinomas. In all positive tumours, both immunostained and unstained areas were observed.

Omission of the critical steps in the immunostaining procedure yielded negative results; no non-specific staining was observed. A strong staining reaction was evident in the liver specimen serving as a positive control. The distribution of CYP2E1 within the liver, with a perivenous zonation phenomenon, was characteristic for this enzyme [9]. Good agreement was observed between the staining results (IRS values) obtained in the tumour samples with the normal and intensified staining procedures (data not shown).

The only structures of normal bronchial tissue that consistently showed immunoreactivity for CYP2E1 were

Table 1 Cell and tissue immunoreactivity for cytochrome P450(CYP)2E1 in normal bronchial tissue (– no immunoreactivity, (+) inconclusive result, + weak immunoreactivity, ++ moderate immunoreactivity, +++ strong immunoreactivity)

Cell or tissue	Immunoreactivity
Bronchial epithelium	
ciliated cells	+/++/+++
goblet cells	–
Seromucous glands	
serous glands	(+)
mucous glands	++
secretory ducts	–/+
Smooth muscle	–/+
Inflammatory cells	
neutrophils	–/++
lymphocytes	–
monocytes	+
plasma cells	–/+
Cartilage	
chondrocytes	+
matrix	–
Submucosal connective tissue	–

Table 2 Immunostaining results for CYP2E1 in normal bronchial tissue and primary pulmonary carcinomas (*IRS* immunoreactivity score)

* The difference in the IRS between adenocarcinomas and squamous cell carcinomas is not statistically significant
[a] See text for details
[b] In 11 of these 13 cases, CYP2E1 was also present in normal bronchial tissue

Tissue	Number of negative cases (IRS 0–2)	Number of positive cases (IRS ≥3)	Proportion of positive cases
Normal bronchial tissue			
seromucous glands	9	19	19/28 (68%)
bronchial epithelium	10	18	18/28 (64%)
Tumour tissue*			
Squamous cell carcinoma	4	9	9/13 (69%)
Adenocarcinoma	6	2	2/8 (25%)
Others[a]	5	2	2/7 (29%)
Total	15	13[b]	13/28 (46%)

seromucous glands and bronchial epithelium. Seromucous glands showed expression of CYP2E1 (IRS ≥3/9) in 19 and bronchial epithelium in 18 of the 28 cases of normal bronchial tissue (Table 2, Fig. 1). The mucous glands were more strongly stained than the serous glands. There was no significant difference in the IRS for seromucous glands between non-drinkers and moderate drinkers (P=0.10 by Mann-Whitney test).

In bronchioli, a weak or moderate immunostaining for CYP2E1 was observed in the ciliated epithelial cells in all of the 11 samples of peripheral lung tissue studied. Weak immunostaining was seen in the few non-ciliated bronchiolar epithelial cells (Clara cells) identified.

Thirteen of the corresponding 28 cases of primary pulmonary carcinoma showed staining for CYP2E1 (Table 2, Figs. 2–4). In 11 of these 13 cases, CYP2E1 was also present in normal bronchial tissue (seromucous glands or bronchial epithelium or both). No statistically significant difference in the IRS was observed between adenocarcinomas and squamous cell carcinomas (P=0.11 by Mann-Whitney test).

No association was observed between the expression of CYP2E1 in tumour tissue and normal bronchial tissue, represented by seromucous glands [r=0.29, P=not significant (NS)]. However, there was a significant correlation between the expression of CYP2E1 in seromucous glands and bronchial epithelium (r=0.61, P<0.01) of normal tissue.

Using the data from our previous study on the expression of CYP3A in the lung [11], the expression of CYP2E1 was compared to that of CYP3A in the 28 patients included in both studies. There was no association between the expression of CYP2E1 and CYP3A in either tumour tissue (r=0.11, P=NS) or normal tissue, represented by seromucous glands (r=0.08, P=NS).

Discussion

In this immunohistochemical study we demonstrated the presence of CYP2E1 in both normal and cancerous lung tissue. About half of the tumours and two-thirds of the corresponding samples of normal bronchial tissue showed expression of CYP2E1. CYP2E1 was expressed in the majority (69%) of the squamous cell carcinomas but only in 25% of the adenocarcinomas studied. However, no statistically significant difference in the immunoreactivity score was observed between adenocarcinomas and squamous cell carcinomas.

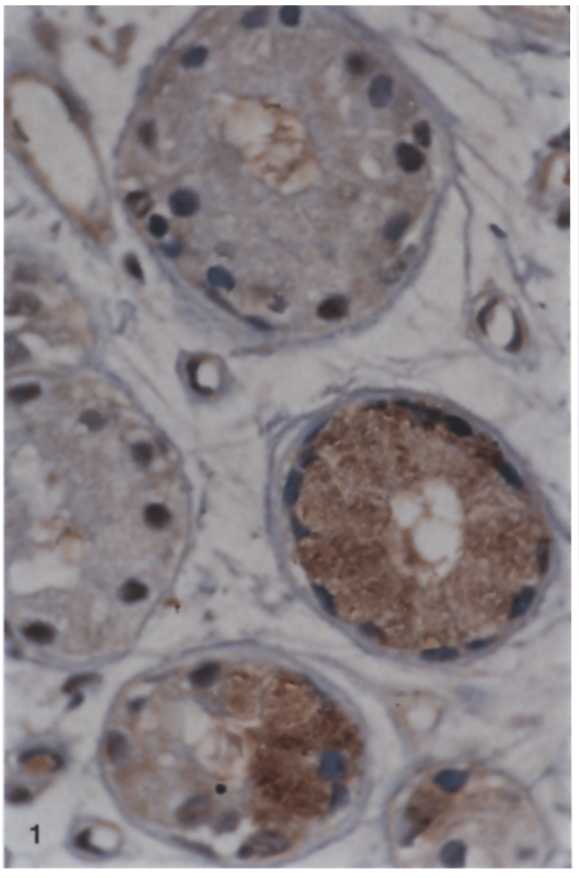
CYP2E1 was also present in bronchiolar epithelial cells; however, it was difficult to differentiate the non-ciliated (Clara) cells from the ciliated ones. This was partly due to the use of paraffin-embedded tissue and an unavoidable incubation step in a microwave oven. Plastic-embedded material may be more suitable for this purpose, and this possibility is currently under investigation in our laboratory.

Our results extend the preliminary findings of Wheeler et al. [17] concerning the expression of CYP2E1 in normal human lung tissue. Using a monoclonal antibody and solid phase radioimmunoassay, these investigators demonstrated the presence of a CYP, immunochemically related to human liver CYP2E1, in human lung microsomes of all eight lung samples studied. The levels of this enzyme were much lower than levels in human liver microsomes. However, western blot analysis of human lung microsomal CYP2E1 did not give a clear positive result in this study [17].

Toussaint and co-workers [15] have recently characterized xenobiotic-metabolizing enzyme systems in human non-small cell lung tumours and the corresponding non-tumour lung tissues in 12 patients. Several major CYP enzymes, including 1A, 2B, 2E1 and 3A4, were assayed by western blot analysis. CYP1A was present in both tumour and non-tumour tissue, CYP1A levels being significantly lower in tumours compared to matched non-tumour tissue. However, none of the other probed CYP enzymes were detected in either tumour or non-tumour lung tissue [15].

In light of the results obtained by Wheeler et al. [17] and Toussaint et al. [15], it seems that, due to expression of CYP2E1 in specific cell types and the presumably low amounts of this enzyme, western blot analysis is not suitable for detecting CYP2E1 in the lung. In contrast, immunohistochemical methods enable detection of small quantities of CYP enzymes as well as localization of these enzymes in specific cells.

As with CYP3A in our earlier study [11] there was no correlation between the expression of CYP2E1 in tumours and normal bronchial tissue. There is also other evidence showing that expression of CYP enzymes may differ in normal and cancerous lung tissue; McLemore et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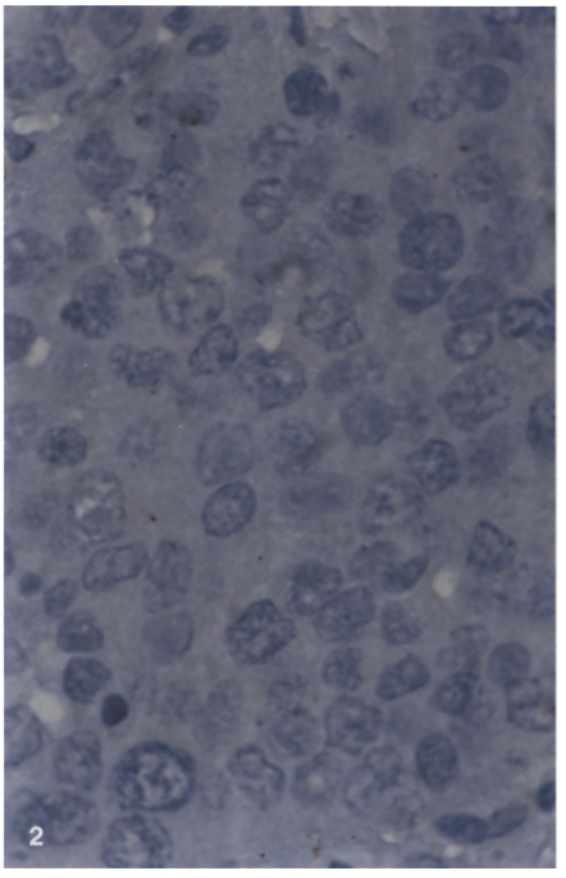
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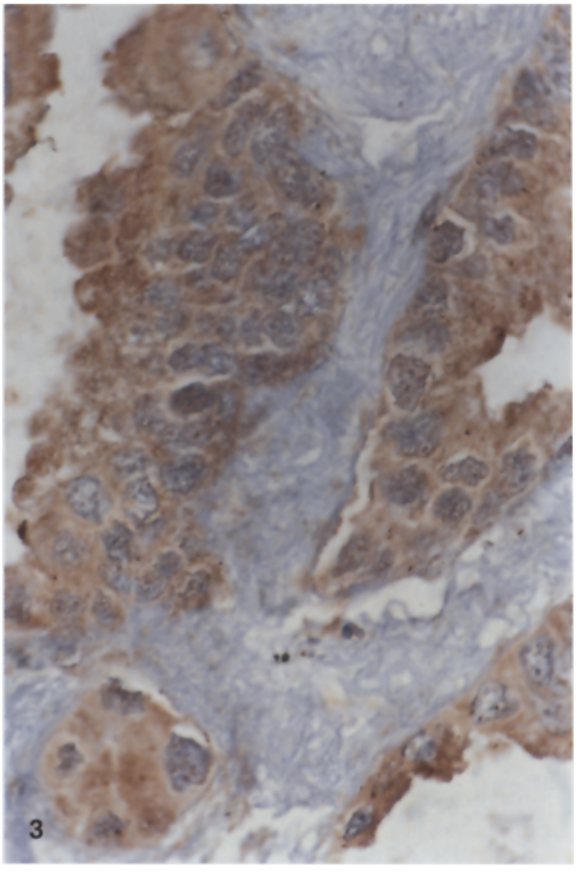
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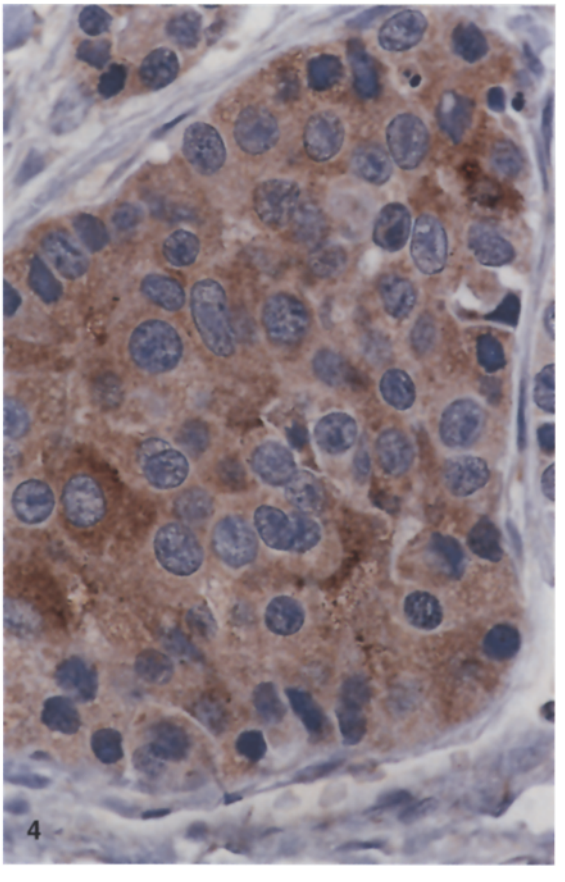
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al. [14] found no positive association between CYP1A1 mRNA levels in paired samples of normal lung tissue and tumour tissue from patients with lung cancer.

The recent study of Czerwinski et al. [3] provides additional information about the expression of CYP enzymes in normal and cancerous lung tissue. These investigators studied the expression of CYP2B7 and CYP4B1 in normal human lung and lung tumours, using the technique of RNase protection. The mRNAs of both of these CYP enzymes were detected in all the normal and a majority of neoplastic tissues, but the levels of expression of the two CYP mRNAs were reduced, about two-fold, in the tumours when compared with normal lung [3].

An additional goal of this study was to compare the expression of CYP3A and CYP2E1 in the lung in the same set of patients. In our recent immunohistochemical study [11], CYP3A was present in 25% of the 32 primary pulmonary carcinomas studied and in a slightly larger proportion of the samples of surrounding normal bronchial tissue. The present study comprised 28 of these patients and showed that CYP2E1 was much more frequently expressed than CYP3A in both normal and cancerous lung tissue. Moreover, there was no association between the expression of CYP2E1 and CYP3A in either tumour tissue or normal tissue, as analysed by Spearman's rank correlation coefficient. Apparently, these CYP enzymes are expressed independently of each other in lung tissue.

In conclusion, CYP2E1 was expressed in a large proportion of both the primary pulmonary carcinomas and corresponding samples of normal bronchial tissue studied. The observed lack of correlation between the expression of CYP2E1 in tumours and normal bronchial tissue suggests, taken together with the findings from other studies, that expression of CYP enzymes is different in normal and neoplastic lung. As CYP2E1 can activate several procarcinogens, the extent of expression of CYP2E1 might influence an individual's susceptibility to lung cancer. Knowledge of the CYP enzyme systems in the lung increases rapidly, and eventually it may be possible to design anticancer agents that are preferentially metabolized, and bioactivated, in pulmonary carcinomas.

Acknowledgements This work was supported by the Dr. Mildred Scheel Foundation (grants W87/91/To1 and W90/92/Ei2), Robert Bosch Foundation, Alexander von Humboldt Foundation (KTK), and by the Ministere de l'Enseignement Superieur et de la Recherche and Rhone Poulenc Rorer through the Bioavenir programme.

Fig. 1 Immunostaining for cytochrome P450(CYP)2E1 in serous and mucous glands of normal bronchial tissue (immunostaining is localized primarily in the mucous glands). ×900

Fig. 2 Squamous cell carcinoma of lung without immunostaining for CYP2E1. ×900

Fig. 3 Strong immunoreactivity for CYP2E1 in all tumour cells of an adenocarcinoma of lung. ×900

Fig. 4 Squamous cell carcinoma of lung showing strong immunostaining for CYP2E1 in all tumour cells. ×900

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