Expression of xenobiotic-metabolizing enzymes in human pulmonary tissue: possible role in susceptibility for ILD

J. Hukkanen*, O. Pelkonen*, H. Raunio*,#

ABSTRACT: The lung is a major target for all inhaled toxicants. Many inhaled chemicals are not hazardous as such, but are biotransformed to reactive intermediates. Therefore, the pathogenesis of interstitial and other lung diseases is intimately linked to exposure to environmental and other chemicals, which may be causative or modifying factors in the cellular pathways and mechanisms mediating oxidative stress and cell protection in the pulmonary tissue.

Several different xenobiotic-metabolizing cytochrome P450 (CYP) and phase II enzymes (i.e. conjugation enzymes including several transferases) are present in the human lung and lung-derived cell lines, possibly contributing to in situ activation and inactivation of chemical toxicants. This paper describes the expression and localization of individual CYP-forms in the lung.

Interindividual differences in the expression of these enzymes may contribute to the risk of developing interstitial and other lung diseases initiated by agents that require metabolic activation.


The lung is a major target for all inhaled toxicants. Many chemicals are not hazardous as such, but are biotransformed to reactive intermediates, often by enzymes in the cytochrome P450 (CYP) superfamily [1]. The same chemicals are also detoxified by catalysis via these enzymes. For example, of the several thousands of compounds present in tobacco smoke, several have been found to undergo metabolic activation through xenobiotic-metabolizing CYP enzymes [2, 3].

Enzymes activating or detoxifying environmental chemicals are not the sole factors in the aetiology of chemical-induced lung diseases. Pro-oxidant and antioxidant enzymes and chemicals (many xenobiotic-metabolizing enzymes have these same properties), as well as various repair systems, also play an important role in the aetiology or modification of diseases. Their possible role in interstitial lung disease (ILD) induced by exogenous agents is outlined by Nemery et al. [4] in this Supplement.

Metabolic capacity of pulmonary cells

A key question concerning organ-specific chemical toxicity is whether the actual target tissue has the capacity to activate (or efficiently inactivate) chemicals [5]. Animal models show that in the case of pulmonary toxicity, several target cells in the lung have the capacity to convert chemicals to reactive forms as well as to detoxify them [6]. Finding evidence for this in humans is more difficult, but various lines of research have established that whole lung tissue, as well as several cell types in the lung, possess metabolic capacity towards numerous xenobiotics [7]. Although the lung contains several enzymatic pathways capable of xenobiotic metabolism, it is generally agreed that the CYP superfamily of enzymes is the main system catalyzing the oxidative metabolism and metabolic activation of most toxicants. However, from the susceptibility point of view, it is equally important to measure variations in phase II detoxifying enzymes (i.e. conjugation enzymes, such as uridine diphosphate (UDP)-glucuronyl transferases and glutathione S-transferases) and pro-oxidant and antioxidant systems, although these areas are less well characterized. It is also notable that, like most of the polycyclic aromatic hydrocarbons (PAH), such as benzo(a)pyrene, the inhaled, highly lipophilic compounds have longer retention times and higher local doses in pulmonary epithelium than less lipophilic compounds, indicating that at least these lipophilic substances are primarily site-of-entry toxicants [6].

Expression of cytochrome P450 forms in human lung

For the most recent update on CYP enzymes, consult [8].

The detection of individual CYP forms in human
lungs by conventional methods, such as protein purification, catalytic activity studies, and Western immunoblotting, has been difficult due to the low abundance of CYPs in lungs [9]. With the advent of the reverse transcriptase-polymerase chain reaction (RT-PCR) technology it has become possible to detect minute amounts of messenger ribonucleic acid (mRNA) in tissue samples. RT-PCR is extremely sensitive and results obtained with it cannot be regarded as a direct indication of the existence of corresponding proteins. Rather, RT-PCR is valuable as a screening method, revealing mRNA that can potentially be translated to functional protein in a given tissue. Conversely, absence of mRNA in RT-PCR analysis is a strong indication of the lack of a corresponding protein product at biologically meaningful levels. Expression of various CYP enzymes in human lung at mRNA and protein level is summarized in Table 1.

CYP1A1 is by far the most studied human pulmonary CYP [10]. The first report on the expression of CYP1A1 mRNA in human lung was by OMIECINSKI et al. [11] in 1990. Soon after, the induction of CYP1A1 mRNA by tobacco smoking [12], the expression of CYP1A1 protein in human lung [13] and the localization and induction of CYP1A1 protein by tobacco smoke [14] were reported. CYP1A1 protein is only detected in smokers [14] and CYP1A1 expression is positively correlated with aryl hydrocarbon hydroxylase (AHH) activity in human lung tissue [15, 16].

Another PAH-metabolizing CYP, CYP1A2, was detected recently by RT-PCR and Western blot in peripheral lung [17], but earlier reports do not corroborate this finding [13, 18–21]. Also, the presence of CYP1B1 protein in the lung is controversial [22, 23]. CYP1B1 mRNA is expressed in human lung [24–26] and it is inducible by smoking [27]. CYP1B1 is also induced by smoking in alveolar macrophages [28].

CYP2A6 mRNA was detected in bronchial epithelium [17, 29], but two reports on the expression of the CYP2A6 protein are contradictory [17, 18]. Studies by the author did not demonstrate CYP2A6 mRNA in whole lung tissue homogenate, probably due to dilution of bronchus-specific mRNA expression with other cell types of the lung [30]. The expression of pulmonary CYP2A6 protein would be of utmost interest because CYP2A6 has a crucial role in the activation of 4-methylnitrosamino-1,3-pyridyl-1-butane (NNK), the tobacco-specific procarcinogen [31].

The CYP2B6 gene is expressed in human lung as a splicing variant called CYP2B7 [21, 27, 32] and the corresponding protein is also expressed [17, 33, 34]. A recent immunohistochemistry study suggests a cell-specific expression of CYP2C proteins only in the serous cells of bronchial glands [35]. Out of four previous Western blot studies, two support [19, 36] and two oppose the expression of CYP2C proteins in human lung [37, 38]. CYP2C8 and CYP2C18 mRNAs have also been detected in the lung [17].

There has been great interest in studying expression of pulmonary CYP2D6 due to its alleged role in the activation of the tobacco-specific procarcinogen NNK [30]. However, although it is the focus of numerous studies [17, 19, 21, 39–41], the data on the expression of CYP2D6 in human lung is inconsistent. The expression of pulmonary CYP2E1 mRNA and protein have been established in several studies [17, 19, 21, 29, 37, 42, 43]. CYP2E1 is an interesting CYP form because it is the most active CYP enzyme in forming oxygen radicals, causing tissue injury [44]. The expression of CYP2F1 has been detected at the mRNA level [21, 24, 45], but there are no published results on the expression of CYP2F1 protein. Recombinant CYP2F1 is capable of activating the pulmonary toxicant 3-methylindole [46].

Several studies have demonstrated the expression of CYP3A protein in human lung [17, 19, 20, 37, 47, 48]. The main pulmonary CYP3A form is CYP3A5 [17, 21, 48, 49]. There are no published results on the expression of CYP4B1 protein, but mRNA is expressed in human lung [21, 24, 32, 50]. It has been speculated that native human CYP4B1 enzyme is not functional due to inability to incorporate haeme [51].

In conclusion, at least CYPs 1A1 (in smokers), 2B7, 2E1 and 3A5 proteins are expressed in human lung. Other CYP forms are also likely to be expressed, but their expression is probably very low or restricted to specific cell types or individuals. Studies on the expression of certain CYP forms (CYP2F1 and CYP4B1) are still required.

<table>
<thead>
<tr>
<th>CYP</th>
<th>mRNA</th>
<th>Protein</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>+++</td>
<td>+++</td>
<td>Smokers</td>
</tr>
<tr>
<td></td>
<td>+/-</td>
<td>-</td>
<td>Nonsmokers</td>
</tr>
<tr>
<td>1A2</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>1B1</td>
<td>++</td>
<td>+/-</td>
<td>Protein in alveolar macrophages</td>
</tr>
<tr>
<td>2A6</td>
<td>++</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>2B6/7</td>
<td>+++</td>
<td>+++</td>
<td>Protein in serous cells of bronchial glands</td>
</tr>
<tr>
<td>2C</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>2D6</td>
<td>+/-</td>
<td>+/-</td>
<td></td>
</tr>
<tr>
<td>2E1</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>2F1</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td>+/-</td>
<td>+</td>
<td>Protein in ~20% of cases</td>
</tr>
<tr>
<td>3A5</td>
<td>+++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>3A7</td>
<td>+/-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4B1</td>
<td>+++</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

CYP: cytochrome P450; mRNA: messenger ribonucleic acid. --: moderate negative evidence; -: weak negative evidence; +/-: conflicting evidence; +: weak positive evidence; ++: moderate positive evidence; +++: strong positive evidence.

Localization of individual cytochrome P450 forms in the lung

The cell-specific localization of individual CYP enzymes in the lung is still largely unknown, since there are only a handful of good immunohistochemical studies concerning CYP forms in human lung. It would be of great benefit to the understanding
of cell-specific toxicity to have a comprehensive picture of localization of different CYP forms. The overall distribution of all CYP enzymes can be estimated from the immunohistochemical distribution of reduced nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase, which is detected in bronchial and bronchiolar epithelium, Clara cells, alveolar lining cells and alveolar macrophages [52].

According to immunohistochemistry, CYP1A1 is mainly expressed at the epithelium of the peripheral airways. CYP1A1 expression does not extend to the epithelium of bronchi >1 mm in diameter and is expressed only in the lungs of smokers [14]. Alveolar macrophages do not express CYP1A1 [14, 28]. Induced CYP1A1 may be a precondition for the development of peripheral lung cancer in smokers, as not a single case of this disease with noninducible CYP1A1 in the lung has been found, and, furthermore, CYP1A1 is localized in the part of the airways in which peripheral cancers arise [14, 53]. A study on the localization of CYP1A1 mRNA by in situ hybridization corresponded well with the protein data [34].

Immunohistochemical analysis with CYP3A5-specific antibody shows that CYP3A5 protein is present in all lung samples studied and is localized to the bronchial, bronchiolar and alveolar epithelium, as well as endothelium and alveolar macrophages [49]. Compared with CYP1A1 localization, CYP3A5 expression extends up to larger bronchi. The highest CYP3A5 level is detected in the bronchial lung. Also, CYP3A4 protein is found in some cell types in a minority (~20%) of lung samples with CYP3A4 specific antibody [49]. A few studies with nonspecific CYP3A antibody also show the same pattern of expression as CYP3A5 antibody [47, 48].

CYP2E1 is also localized to human bronchial, bronchiolar and alveolar epithelium, and alveolar macrophages [33, 42, 43]. The localization of the highest expression cannot be evaluated since none of these studies examined both bronchial and peripheral lung. One immunohistochemical report localizes CYP2B7 to human Clara cells [33] and a recent study by YOKOSE et al. [35] observed CYP2C protein in serous cells of bronchial glands, but not in any other cell type of the lung.

**Surrogate tissues**

Various surrogate tissues have been used in human biomonitoring studies, the most popular being peripheral blood lymphocytes [55]. Mitogen-treated lymphocytes display inducible CYP1A1 and formation of benzo(a)pyrene-deoxyribonucleic acid (DNA) adducts, and it is suggested that these parameters may correspond with those of the lung tissue in the same individual, although this issue is controversial and still not definitively settled [5, 9]. Alveolar macrophages, which presumably originate from peripheral blood monocytes, have an important role as defence cells against inhaled particles. The expression pattern of CYP genes in alveolar macrophages resembles the pattern found in the whole lung tissue [21]. Thus, alveolar macrophages may provide a model for investigating the relationship between the capacity to activate and detoxify chemicals in an easily accessible lung cell type [28]. In these cells, at least CYP1B1, CYP2E1 and CYP3A5 proteins are expressed, while CYP1A1 is not [15, 28, 42, 49].

**Inducibility of cytochrome P450 forms in the lung**

CYP1A1 is induced by tobacco smoking in human lung, and there is also evidence for the induction of CYP1B1 in smokers [14, 27, 28]. Information about induction of other pulmonary CYP forms in vivo does not exist. However, studies in a continuously growing pulmonary adenocarcinoma cell line (A549) indicate that CYP3A5 is inducible by glucocorticoids [56]. CYP1A1, CYP1B1 and CYP3A5 participate in the activation of PAHs in the tobacco smoke [10, 57, 58] and thus, their induction could have practical significance.

**Genetic differences in the pulmonary expression of cytochrome P450s**

The expression of CYP1A1 is regulated by genetic polymorphism and it has been claimed that this hereditary variability contributes to individual susceptibility to environmental chemicals [59]. Although the interindividual variability of CYP3A5 expression in the liver and lung is rather large, several 10-fold, relative contributions of environmental and genetic factors have not been elucidated. “Classical” polymorphic enzymes CYP2D6 and CYP2C19 have not been demonstrated in the human lung. Among phase II enzymes, at least glutathione S-transferase M1 (GSTM1) has been shown to be polymorphic in human lungs and may be associated with differential susceptibility to lung cancer [53, 60].

**Conclusions**

The patterns of constitutive and induced expression of cytochrome P450 forms in several relevant human pulmonary cell types are being unravelled with increasing precision. However, knowledge of the functional activities and cell-specific localization of the pulmonary cytochrome P450 forms is still incomplete, mostly due to sensitivity problems associated with measurements in tissues with a low abundance of cytochrome P450 enzymes. In addition, very little is known about the consequences of interindividual variations in both basal and induced pulmonary cytochrome P450 expression. Reactions catalyzed by cytochrome P450 enzymes are often "leaky", resulting in the transfer of electrons to produce oxygen radicals. Cytochrome P450-catalyzed reactions can thus produce xenobiotic metabolites and oxygen species capable of interfering with cell homeostasis. Thus, the role of xenobiotic metabolism catalyzed by individual pulmonary cytochrome P450 forms in the aetiology of diseases such as interstitial
lung diseases, lung cancer and chronic obstructive pulmonary disease, is still elusive and an intriguing subject for future studies.

References


