Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental and Toxicologic Pathology 57 (2005) 189–204
EXPERIMENTAL AND TOXICOLOGIC PATHOLOGY
www.elsevier.de/etp

Metabolism and bioactivation of toxicants in the lung. The in vitro cellular approach

José V. Castell[a,b,*], M. Teresa Donato[a,b], María José Gómez-Lechón[a]

[a]*Research Center, University Hospital La Fe, Avda de Campanar 21, E-46009 Valencia, Spain*
[b]*Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Valencia, Valencia, Spain*

Received 25 April 2005

Abstract

Lung is a target organ for the toxicity of inhalated compounds. The respiratory tract is frequently exposed to elevated concentrations of these compounds and become the primary target site for toxicity. Occupational, accidental or prolonged exposure to a great variety of chemicals may result in acute or delayed injury to cells of the respiratory tract. Nevertheless, lung has a significant capability of biotransforming such compounds with the aim of reducing its potential toxicity. In some instances, the biotransformation of a given compound can result in the generation of more reactive, and frequently more toxic, metabolites. Indeed, lung tissue is known to activate pro-carcinogens (i.e. polycyclic aromatic hydrocarbons or *N*-nitrosamines) into more reactive intermediates that easily form DNA adducts.

Lungs express several enzymes involved in the metabolising of xenobiotics. Among them, cytochrome P450 enzymes are major players in the oxidative metabolism as well metabolic bioactivation of many organic toxicants, including pro-carcinogens. Xenobiotic-metabolising P450 enzymes are expressed in bronchial and bronchiolar epithelium, Clara cells, type II pneumocytes, and alveolar macrophages Individual CYP isoforms have different patterns of localisation within pulmonary tissue. With the aid of sensitive techniques (i.e. reverse transcriptase-polymerase chain reaction, RT-PCR) it has become possible to detect CYP1A1, CYP1B1, CYP2A6, CYP2B6, CYP2E1 and CYP3A5 mRNAs in lung cells. Less conclusive results have been obtained concerning CYP2Cs, CYP2D6 and CYP3A4. CYP3A5 protein appears to be widely present in all lung samples and is localised in the ciliated and mucous cells of the bronchial wall, bronchial glands, bronchiolar ciliated epithelium and in type I and type II alveolar epithelium.

Lung cells also express Phase II enzymes such as epoxide hydrolase, UGT1A (glucuronyl transferase) and GST-P1 (glutathione *S*-transferase), which largely act as detoxifying enzymes. A key question concerning organ-specific chemical toxicity is whether the actual target has the capacity to activate (or efficiently inactivate) chemicals. Results of several studies indicate that the different xenobiotic-metabolising CYPs, present in the human lung and lung-derived cell lines, likely contribute to in situ activation of pulmonary toxins, among them, pro-carcinogens. Some CYPs, in particular CYP1A, are polymorphic and inducible. Interindividual differences in the expression of these CYPs may explain the different risk of developing lung toxicity (possibly cancer), by agents that require metabolic activation.

Few cell lines, principally A549, have been used with variable success as an experimental model for investigating the mechanisms of toxicity. Although RT-PCR analysis has evidenced the presence of the major human pulmonary CYP mRNAs, the measurable P450 specific activities are, however, far below those present in human lungs. Detection of the toxicity elicited by reactive metabolites requires the use of metabolically competent cells; consequently, better

*Corresponding author. Research Center, University Hospital La Fe, Avda de Campanar 21, E-46009 Valencia, Spain.
E-mail address: jose.castell@uv.es (J.V. Castell).

0940-2993/$ - see front matter
doi:10.1016/j.etp.2005.05.008

performing cells are needed to ensure realistic in vitro prediction of toxicity. Genetic manipulation of lung-derived cells allowing them to re-express key biotransformation enzymes appear to be a promising strategy to improve their functionality and metabolic performance.

Keywords: Lung; Pulmonary toxicity; CYP; Bioactivation; A549 cells

Cellular and biochemical principles of lung toxicity

Inhalation represents one of the major routes by which the body enters in contact with gases, as well as solids and liquids suspended in the air. Once these compounds have entered the respiratory tract, inhaled materials may be absorbed and interact with lung cells or the surrounding tissues. Many of these chemical substances pose a risk to the lung as they include specific pneumotoxins and carcinogens. The respiratory tract is, therefore, a primary target organ for toxic inhaled compounds. Some examples of harmful organic chemicals to which humans are accidental or occupationally exposed by inhalation include polycyclic aromatic hydrocarbons, aromatic amines, halogenated compounds, aliphatic compounds, aldehydes, and ketones. Many of those compounds can be found in significant concentrations in the air of urban and industrial settings (either the gas/vapour state or adsorbed onto respirable particles), and in cigarette smoke (Dybdahl et al., 2004; Hecht, 1999; Karlsson et al., 2005). Our knowledge about the potential toxicity of many chemicals present in our environment is quite limited. A significant number of chemicals produced in the range 2–100 ton/year and used in industrial processes are insufficiently known in terms of their potential toxicity. Among them, there are many compounds for which the available toxic information and potential human health risks associated with their inhalation is almost inexistent. Awareness on this problem has been raised by the European Union, by promoting the REACH-program (REACH, 2003).

Situated at the air/blood interface, lungs are a prominent target organ for numerous types of chemically induced damage as a result of exposure to xenobiotics after inhalation or following systemic administration and accumulation in the lungs. The mechanisms by which xenobiotics cause damage to pulmonary tissue is a complex and partially characterised issue. Complexity arises because of the heterogeneity of the lung, containing over 40 different cell types, and the remarkable variety in cell functionality (Yost, 1999). Functions range from gas exchange by epithelial alveolar cells, secretory cells with cilia, designed to move foreign particles up to tracheobronchial tract, to endothelial cells that line the pulmonary vasculature, providing a junction between bloodstream and respiratory cells. Each of these cells, and the function they sustain, is a potential target for toxic action. The most susceptible lung cells are capillary endothelial cells, pulmonary alveolar macrophages, non-ciliated bronchiolar epithelial cells (Clara cells), ciliated bronchiolar epithelial cells, and type I and type II alveolar epithelial cells (Kehrer, 1993; Hecht, 1999).

The lung has the potential of metabolising many foreign compounds. Biotransformation is the process by which cells modify xenobiotics they enter into contact, with the ultimate goal of facilitating the elimination of lipophillic substances, which otherwise would accumulate in cell's lipids. This is achieved by a set of broad specificity enzymes capable of introducing new functional groups (Phase I reactions), or conjugating with internal cell's molecules, to increase its water solubility (Phase II reactions). Among Phase I enzymes, the hemoproteins encoded by CYP genes (cytochrome P450-depending monooxygenases, in short *P450*) are considered the most actively involved in the biotransformation of xenobiotics. These are enzymes that oxidise substrate, making use of one out of the two atoms of oxygen (monooxygenases), the other one being reduced to H_2O by NADPH (Testa, 1995).

A complete understanding of how a compound may induce cell damage is lacking. Reviews addressing the various pathological and biochemical changes induced by lung toxins have been published elsewhere (Gram, 1997; Hecht, 1999). Emerging among them is the cumulative experimental evidence of the role of xenobiotic metabolism as a cause of lung toxicity.

In general, biotransformation reactions are beneficial in that they help the pulmonary tissues to eliminate foreign compounds. Sometimes, however, these enzymes transform an otherwise harmless substance into a reactive form. P450-mediated oxidations frequently result in the generation of more reactive intermediates (i.e. electrophiles), a process known as *metabolic bioactivation* (Fig. 1). Thus, many compounds that elicit toxic injury to the lung are not intrinsically pneumotoxic, but cause damage to target cells following metabolic activation. A classic example is the activation of benzo(*a*)pyrene, which is a constituent of tobacco smoke and combustion products, into reactive forms capable of binding to DNA and eventually leading to cancer formation.

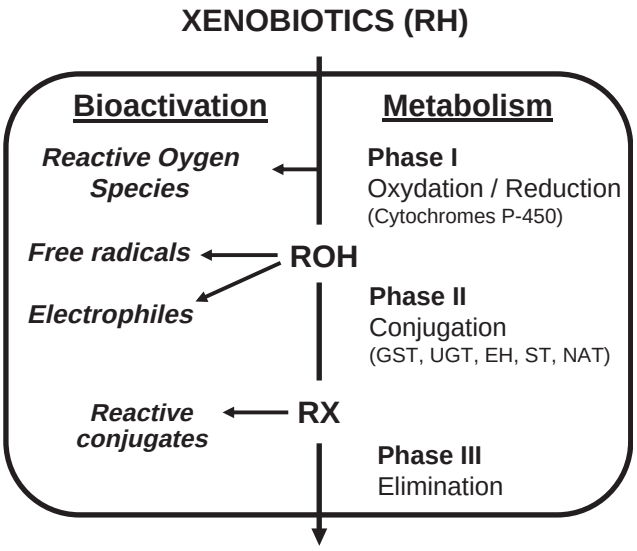

Fig. 1. Metabolism and bioactivation of xenobiotics. Phase I reactions can generate more reactive metabolites than the parent compound. Phase II reactions tend to render more water-soluble and less active metabolites, however, bioactivation can also occurs. GST: glutathione *S*-transferase; UGT: UDP-glucuronyltransferase; EH: epoxide hydrolase; ST: sulphotransferase; NAT: *N*-acetyltransferase.

As the resulting intermediates are highly reactive and unstable, they tend to exert toxicity in situ rather than in distant tissues. The site and importance of the pulmonary injury ultimately depends upon factors such as the concentration of the reactive agent within lung, the existence of an adequate pulmonary detoxication pathway, and the bioactivation to a more toxic species. These factors also determine which specific type of lung cells will be preferentially damaged (Boyd, 1984). Toxicity in the target cell occurs when metabolic activation is followed by inappropriate fate (*detoxification*) of the reactive metabolite, which is normally achieved by Phase II reactions. In such cases, toxicity reflects the unbalance between activation and detoxification reactions (Ding and Kaminsky, 2003).

Assessment of the potential toxicity of new chemicals for man is still largely based on data obtained from studies with experimental animals. However, the well-known species differences in biotransformation enzymes raises concerns about the suitability of animal data for predicting lung toxicity in humans as a result of *bioactivation* reactions (Bond, 1993). The development of human relevant in vitro models (i.e. cell lines from human pulmonary origin), can notably contribute to better understanding the basis of chemical-induced lung toxicity in man and, in particular, the role of pulmonary biotransformation enzymes in the bioactivation/detoxication processes.

Xenobiotic-metabolising enzymes in human lung

Biotransformation of xenobiotics enables the elimination of lipophillic substances that otherwise might accumulate in tissues, thereby causing toxic effects. The process involves different chemical modifications of the compounds resulting in the appearance of more polar and reactive functional groups in the molecule (Phase I reactions), followed by conjugation with endogenous molecules such as glucuronic acid, glutathione, sulphate and amino acids (Phase II reactions). Most Phase I reactions consists in oxidation reactions catalysed by two groups of monooxygenases, i.e. cytochrome P450-depending monooxygenases (encoded by the CYP genes) and flavin monooxygenases (FMN), as well as reductions catalysed by P450 reductase and other reductases.

Expression of P450 enzymes in pulmonary tissue

The proteins encoded by the CYP superfamily (P450 monooxygenases) are the responsible of the oxidative metabolism of the vast majority of xenobiotics (drugs, environmental pollutants, carcinogens), as well endogenous compounds. The human P450 system consists of ca. 50 isoforms that exhibiting major differences with respect to their catalytic specificity and tissue pattern of expression. P450 enzymes act as monooxygenases, i.e. use one atom of molecular oxygen to oxidise xenobiotics, and requiring the aid of NADPH-cytochrome P450 reductase, to provides the electrons required for the reduction of the second oxygen atom to H_2O. The presence and adequate functionality of the reductase is determinant for effective xenobiotic oxidation by P450 enzymes.

P450 enzymes are grouped in families and subfamilies according to their amino acid sequence homology (Fig. 2). Families CYP1, CYP2, and CYP3 are the main player in the oxidative metabolism of xenobiotics. Despite liver is the organ with the highest expression of most CYP isoforms other tissues have its own particular profile of P450 enzyme expression, what ultimately determine its capability of xenobiotic biotransformation (and bioactivation).

The limited availability of suitable human tissue for experimental studies and the low P450 expression in human lung, compared with that found in rodent species (Wheeler et al., 1990), have notably difficult the identification of the lung P450 isoforms. By means of molecular biology techniques was possible to qualitatively demonstrate the presence of several CYP mRNAs in human lung cells, namely CYP1A1, 1A2, 1B1, 2A6, 2A13, 2B6, 2Cs, 2D6, 2E1, 2F1, 2S1, 3A4, and 3A5 (Wheeler et al., 1990, 1992; Shimada et al., 1996; Hukkanen et al., 2002). Immunohistochemical analysis

has evidenced significant expression of P450 enzymes in different pulmonary cells including alveolar type I and type II cells, Clara cells, ciliated columnar epithelial cells, and macrophages (Kim et al., 2004).

The vast majority of published data on CYP expression in the human lung is qualitative. Quantitative real-time RT-PCR methods can now be used for a precise and reproducible quantitative assessment of CYP mRNAs in tissues with very low P450 expression as the lung. The procedure is based on the use of internal standards that are co-amplified in the course of the polymerase chain reaction, allowing to precisely determine the amount of a given mRNA in a sample (Perez et al., 2003; Nishimura et al., 2003). As shown in Table 1 the analysis of the various CYP mRNAs in a series of human lung tissues revealed interesting features. The most abundant mRNAs were CYP1B1, 2B6, 2C9 and 2E1. Other minor, but measurable CYP mRNAs were CYP1A1, CYP3A4, CYP3A5, and CYP2D6. CYP1A2, CYP2A6 and CYP2A13 were only detected in some donors. No measurable levels of CYP2C19 mRNA were found in any of the eight human lung samples analysed. These results are in agreement with previous data in pooled human lung samples (Nishimura et al., 2003).

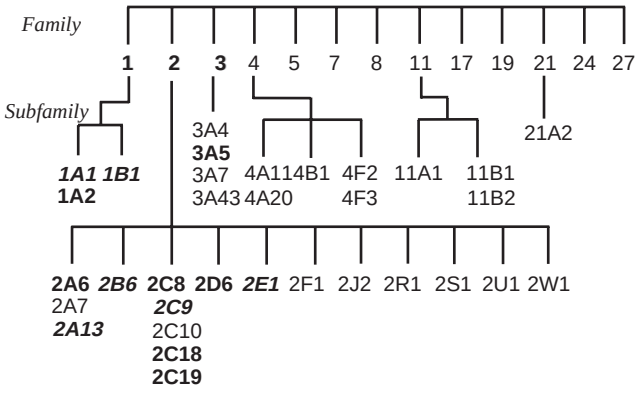

Fig. 2. Human CYP involved in xenobiotic metabolism. The human CYP superfamily comprises ca. 50 genes, according to the mapped human genome. Genes are classified into families and subfamilies according to the degree of nucleotide and amino acid sequence homology. Families 1, 2, and 3 are largely responsible of biotransformation of xenobiotics (bold). Italic typed indicates the predominant enzymes in human lung.

Variability in mRNA among donors was significant for all CYP genes, CYP1A2 and 2A13 showing >100-fold variability. High interindividual differences in levels of CYP1A1 and CYP1B1 proteins were also found by immunohistochemical analysis (Kim et al., 2004).

The evaluation of the metabolic capacity of any tissue does require functional analysis (i.e. enzyme activity level). The activity of each individual P450 is currently done in microsomes obtained from the tissue. Because of the low activities expressed in lung highly sensitive probes need to be used. The *O*-deethylation of 7-ethoxycoumarin (ECOD activity) is a very sensitive fluorimetric method to assess overall P450 activity, as this reaction is catalysed by several human P450s (Waxman et al., 1991; Donato et al., 1999). Table 2 displays the results obtained after measuring ECOD and NADPH-cytochrome P450 reductase activities in human lung microsomes from different donors. Both activities are present in all lung samples studied at levels significantly lower that those reported in the liver (Pearce et al., 1996; Donato et al., 1999), but measurable, reproducible and consistent with previous findings (Ohnhaus and Bluhm, 1987; Wheeler et al., 1990; Czerwinski et al., 1994), indicating an active P450 monooxygenase system is operating in all lung tissue samples.

Evaluation of the activity of individual P450s requires the use of appropriate substrate each one being a specific substrate for a given P450 enzyme. Unfortunately, selective substrates do not exist for all individual P450s. In our study, we made use of ethoxyresorufin

Table 1. CYP mRNA levels in human lung tissue

Lung	CYP1A1	CYP1A2	CYP1B1	CYP2A6	CYP2A13	CYP2B6	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4	CYP3A5
1	0.45	3.0	35.5	0.076	1.78	56.1	3.0	<0.01	0.31	48.3	10.2	5.3
3	16.0	9.8	111	0.19	1.07	24.3	6.7	<0.01	0.40	34.4	9.3	5.3
4	2.4	0.78	25.9	<0.01	1.75	23.9	7.6	<0.01	0.77	9.7	2.1	
5	1.7	<0.01[a]	66.5	<0.01	<0.01	23.3	7.0	<0.01	0.19	8.5	3.3	1.3
6	4.8	2.0	16.6	<0.01	<0.01	66.7	25.5	<0.01	0.42	3.0	10.0	11.4
7	13.3	1.5	71.7	<0.01	<0.01	131	30.7	<0.01	0.19	8.2	15.2	12.2
8	7.1	3.8	58.1	<0.01	<0.01	54.1	22.8	<0.01	0.56	3.5	10.4	2.4
Mean	6.5	—	55.0	—	—	54.1	14.8	—	0.66	35.4	8.6	6.3
SD	6.0		32.2			38.2	11.2		0.60	25.0	4.5	4.5
Fold variation[b]	35	>1000	6	>20	>175	5	9		3	15	6	8

Specific mRNA levels were quantified by quantitative RT-PCR and results are expressed as (CYP mRNA/β-actin mRNA) $\times 10^4$.

[a]Under the limit of detection.

[b](Highest value–lowest value)/lowest value.

(ethoxyresorufin *O*-deethylation, EROD), coumarin (coumarin 7-hydroxylation, COH), benzoxyresorufin (benzoxyresorufin *O*-debenzylation, BROD), pentoxyresorufin (pentoxyresorufin *O*-dependentylation, PROD), testosterone (testosterone 6*β*-hydroxylation, 6*β*-OHT) diclofenac (diclofenac 4′-hydroxylation, D4OH) and chlorzoxazone (chlorzoxazone 6-hydroxylation, C6OH) (Donato et al., 1999). Only COH (CYP2A6), PROD (CYP2B6), 6*β*-OHT (CYP3A), and ECOD (total P450) activities could be detected in all human lung donors (Table 2). Neither D4OH nor C6OH were measurable. Respect to EROD and MROD activities (representative of enzymes belonging to CYP1 family) were undetectable in lung tissue, except in samples from smoker donors (donors 6 and 8).

The cellular localisation of each individual P450 in the lung is poorly known. CYP1A1 is mainly present in bronchiolar epithelium and alveolar cells from smokers (Willey et al., 1997; Saarikoski et al., 1998). Expression of CYP1B1 has been identified in bronchial epithelial cells as well as alveolar macrophages (Willey et al., 1997; Piipari et al., 2000). CYP2A6 mRNA has been found in bronchial epithelial cells (Crawford et al., 1998), whereas CYP2B6 has been detected at protein and/or mRNA levels in bronchial epithelial cells and alveolar type II pneumocytes (Mori et al., 1996; Willey et al., 1997). CYP2E1 is also expressed in bronchial and alveolar epithelial cells and bronchoalveolar macrophages (Mori et al., 1996; Crawford et al., 1998). Immunohistochemical analysis showed CYP3A isoenzymes, mainly CYP4A5, in ciliated and mucous bronchial cells, bronchiolar epithelium, type I and type II alveolar epithelial cells, vascular and capillary endothelium and alveolar macrophages (Anttila et al., 1997). As reported, CYPs are evenly present in the different pulmonary cells types in man. This contrasts with what has been reported in rodents where most P450 activity is limited to Clara and type II alveolar cells. In humans, on the contrary, Clara cells seem to lack smooth endoplasmic reticulum and show a very low expression of P450 enzymes and, consequently, are likely to play a minor role in the bioactivation of xenobiotics (Gram, 1997; Ding and Kaminsky, 2003).

Interindividual variability in P450 activities

Interindividual P450 variability is the consequence of both geno- and phenotypic factors (Fig. 3). The CYP genes encoding P450 enzymes are largely polymorphic.

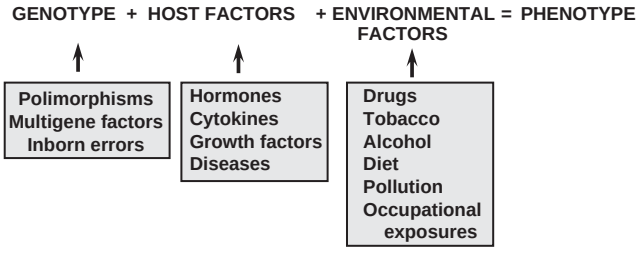

Fig. 3. Determinants of interindividual variability of xenobiotic metabolism. Both genotypic (genetic polymorphisms) as well environmental and physiological factors influence the expression of biotransformation enzymes.

Table 2. Phase I activities in human lung tissue

Lung	P450 reductase	ECOD (several CYPs)	EROD (CYP1A/B)	MROD (CYP1A2)	COH (CYP2A)	PROD (CYP2B6)	D4OH (CYP2C9)	C6OH (CYP2E1)	6*β*OHT (CYP3A)	UGT	GST
2	7.5	2.57	<0.001	<0.001	32.9	0.010	<0.001	<0.001	0.143	13.7	52.7
4	11.0	2.52	<0.001[a]	<0.001	13	1.240	<0.001	<0.001	0.147	210	51.1
5	13.9	2.86	<0.001	<0.001	30.9	0.600	<0.001	<0.001	0.102	29.2	42.4
6	10.9	2.49	<0.001	0.115	18.5	0.445	<0.001	<0.001	0.161		51.3
7	10.8	0.72	<0.001	<0.001	12.5	0.205	<0.001	<0.001	0.107	10.2	40.3
8	13.6	0.99	0.290	0.160	29.1	0.220	<0.001	<0.001	0.113	26.5	60.6
9	10.1	1.71	<0.001	<0.001	44.2	0.005	<0.001	<0.001	0.196	32.1	20.6
10	4.7	1.42	<0.001	<0.001	60.2	0.080	<0.001	<0.001	0.109	15.4	43.3
Mean	10.3	1.91	—	—	30.2	0.351	—	—	0.135	48.1	44.8
SD	3.01	0.81			26.3	0.416			0.033	71.8	11.3
Fold variation[b]	2	3	>290	>160	4	248			2	21	3

P450 reductase: NADPH-cytochrome P450 reductase; ECOD: 7-ethoxycoumarin *O*-deethylase; EROD: ethoxyresorufin *O*-deethylase; MROD: methoxyresorufin *O*-demethylase; COH: coumarin 7-hydroxylase; PROD: pentoxyresorufin *O*-dependentylase; D4OH: diclofenac 4'-hydroxylase; C6OH: chlorzoxazone 6-hydroxylase; 6*β*OHT: testosterone 6*β*-hydroxylase; UGT: UDP-glucuronyltransferase; GST: glutathione *S*-transferase. Activities were measured in human lung microsomes (or in S9 fraction for GST) using selective substrates and expressed as pmol/mg/min, except in the case of P450 reductase and GST which were expressed as nmol/mg/min.

[a]Under the limit of detection.

[b](Highest value–lowest value)/lowest value.

Single nucleotide polymorphisms (SNPs) are known, resulting in changes of enzymatic activity and/or specificity, that are inherited in a mendelian manner. The actual phenotype is the result of the alleles inherited from parents.

Another interesting feature of CYP genes is their ability to be induced by xenobiotics. The transcription of most CYPs can be influenced to a significant extent by the exposure of cells to compounds known as *enzyme inducers*. They belong to different chemical categories and, via nuclear receptors, interact with the promoter regions of several CYP genes activating their transcription. Dietary habits, life style, exposure to pollutants or toxins influence the expression of many P450 enzymes. This phenotypic variability is more pronounced in animals than in man. Enzyme induction results in a more rapid metabolism of the inducer (and other xenobiotics), what in turn may lead to an increased formation of toxicologically active metabolites. Enzyme induction does significantly contribute to interindividual differences in xenobiotic toxicity.

CYP1 genes are the most extensively studied human pulmonary P450 enzymes because of their recognised role in polycyclic aromatic hydrocarbons metabolism and its inducibility by tobacco smoking. Levels of CYP1A1 and CYP1B1 proteins in lung tissue from smokers and ex-smokers are quantitatively greater than in non-smokers (Kim et al., 2004; Willey et al., 1997; McLemore et al., 1990a). Similarly, smoking was found the most important factor related to the presence of aryl hydrocarbon hydroxylase in lung tissue (a CYP1A1-related activity; Anttila et al., 1991). A possible association between interindividual CYP1A1 differences (due to polymorphisms or enzyme induction) and susceptibility to lung cancer has been postulated (Anttila et al., 1991; Bartsch et al., 1992). *Aryl hydrocarbon receptor (AhR)* stimulates transcription of CYP1 genes via direct interaction with the promoter region of the gene. Upon binding of the inducer (i.e. polycyclic aromatic hydrocarbons) to cytosolic AhR, the complex is translocated to the nucleus where it heterodimerises with the *nuclear AhR nuclear translocator (Arnt)*, and binds to an enhancer/promoter DNA region of CYP1 genes (Fig. 4) (Gonzalez and Fernandez-Salguero, 1998; Whitlock, 1999). Both AhR and Arnt proteins are expressed in human lung (Hayashi et al., 1994). The *AhR repressor (AhRR)* has been recently identified as a negative factor that suppresses the AhR function by competing with AhR dimerisation with Arnt (Mimura et al., 1998). AhRR is constitutively expressed in normal human lung and other tissues, and its expression might be affected by smoking or exposure to environmental pollutants (Tsuchiya et al., 2003). AhR and AhRR very likely constitute a regulatory loop of xenobiotic signal transduction, although, this phenomena has not been fully confirmed (Mimura et al., 1998).

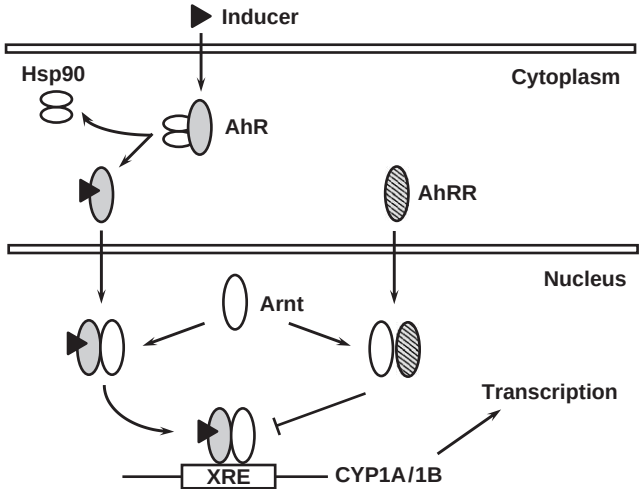

Fig. 4. Mechanism of AhR-mediated CYP1A/1B induction. Upon binding of the inducer to cytosolic AhR (Aryl hydrocarbon receptor), the complex is translocated to the nucleus where heterodimerises with the aryl hydrocarbon receptor nuclear translocator (Arnt). Ligand-activated AhR/Arnt heterodimer transactivate the expression of target genes by binding to xenobiotic response element (XRE). The ArR repressor (AhRR) inhibits AhR function by competing for dimerising with Arnt and binding to the XRE-sequence.

Apart from CYP1 enzymes, there are no conclusive in vivo data about factors and mechanisms controlling the expression of P450 enzymes in human lung. In vivo studies have shown that whereas CYP2B or NADPH-cytochrome P450 reductase expression are not affected by cigarette smoking, CYP2A6 and CYP3A are repressed in certain human lung cells (Willey et al., 1997; Crawford et al., 1998; Piipari et al., 2000). The mechanisms of these effects remain unknown.

CYP3A enzymes are present in different types of human lung cells, CYP3A5 being the predominant form (Raunio et al., 1999; Ding and Kaminsky, 2003). This predominant expression of CYP3A5 in the lung over the most commonly tissue present CYP3A4, appears to be consequence of the existence of tissue-specific upregulating and down regulating transcription factors (Raunio et al., 2005). Both pregnane X receptor (PXR) and constitutive active receptor (CAR) participate in the regulation of CYP2B6, CYP2C, CYP3A4, and CYP3A7 expression (Waxman, 1999). These nuclear receptors share some ligands and a common heterodimerisation partner, the retinoid X receptor (RXR). Upon binding of the ligand and dimerisation with RXR, the heterodimer bind with DNA responsive elements resulting in an activation of gene transcription (Quattrochi and Guzelian, 2001). However, very low or negligible levels of PXR and CAR mRNAs have been found so far in the human lung (Bertilsson et al., 2001; Baes et al., 1994).

Expression of phase II enzymes in pulmonary tissue

The lung also contains Phase II enzymes that play an active role in the elimination of xenobiotics as well their metabolites formed during phase I reactions. Glutathione *S*-transferases (GSTs), UDP-glucuronyltransferases (UGTs) and epoxide hydrolases, have been identified in pulmonary tissue of different laboratory animals (Bond, 1993; Yost, 1999). Noteworthy, the available information in human lung tissue is very limited. Studies on the expression of UGT enzymes in human lung have attracted attention, because pulmonary first-pass metabolism by glucuronidation has been reported to be relevant in laboratory animals (Cassidy and Houston, 1984). Sixteen different human UGT proteins have been identified to date, eight are encoded by the *UGT1A* gene locus (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, and 1A10), while eight are encoded by *UGT2* genes (UGT2A1, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17, and 2B28) (Tukey and Strassburg, 2000). These isoforms exhibit distinct, but frequently overlapping substrate specificity (Batt et al., 1994).

The fluorescent 4-methylumbelliferone is metabolised by multiple human UGT isoforms and is a useful probe for the general assessment of glucuronidation activity (Uchaipichat et al., 2004). Significant levels of 4-methylumbelliferone glucuronidation were found in lung tissue samples (Table 2) but UGT activities are, in general, much lower than in the liver (Yoshimura et al., 1992; Ren et al., 2000). Consequently, the contribution of pulmonary glucuronidation to the overall UGT-mediated metabolism of a xenobiotic is quantitatively less important. No measurable expression of any UGT1A gene has been observed in human lung upon RT-PCR analyses (Ren et al., 2000; Zheng et al., 2002). Controversial findings are, however, reported concerning the UGT2B family. Whereas Turgeon et al. (2001) found detectable levels of UGT2B4, 2B7, 2B10, 2B11, 2B15, and 2B17 mRNAs, other authors did not find the presence of UGT2B4, 2B7, 2B15 and 2B17 (Zheng et al., 2002).

Glutathione transferases (GSTs) are a family of enzymes that catalyse the conjugation of glutathione to a wide variety of endogenous and exogenous electrophilic compounds. GSTs are divided into two distinct superfamily members: (a) the membrane-bound microsomal enzymes, that play a relevant role in the endogenous metabolism of leucotrienes and prostaglandins, and (b) the cytosolic enzymes involved in the defence against reactive chemical agents. Human cytosolic GSTs are polymorphic and can be divided into six classes: alpha, mu, omega, pi, theta, and zeta. GSTs are widely distributed in tissues, being GSTP1 (a pi class enzyme) the most abundant GST isoform in the lung (Wang et al., 2003). Using 1-chloro-2,4-dinitrobenzene, an appropriate substrate for different GST enzymes (Singhal et al., 1992), low but significant activity levels of activity were consistently found in lung tissue from various donors (Table 2). The measured activity is similar to that reported in the rat (Commandeur et al., 1995), but markedly lower than that found in human liver (Donato et al., 1999).

Sulphotransferases are cytosolic enzymes that conjugate xenobiotics with sulphate using phospho-adenosyl phosphopsulphate (PAPS) as donor. They constitute the SULT gene superfamily. Eleven distinct human SULT forms are known, which strongly differ in their tissue distribution and their substrate specificity. Common functionally relevant genetic polymorphisms of the transcribed region are known for two of the forms, SULT1A1 and 1A2. Studies using recombinant test systems demonstrate that many pro-mutagens are activated with high selectivity by an individual SULT form. Pronounced differences in pro-mutagen activation were detected between the different human forms, including their allelic variants, and also between orthologous SULTs from different species (Glatt, 2000).

Metabolic activation of xenobiotics and toxicity to lung cells

Toxicity to lung tissue cells from inhaled or systemically administered compounds frequently occurs after its bioactivation to more reactive compounds. The specific lung cells damaged may depend upon factors such as preferential exposure or accumulation of parent compounds and/or metabolites in a given cells, occurrence of a specific mechanism of activation, or differences in cellular defence mechanisms. The involvement of P450 enzymes in the mechanisms of toxicity of a wide variety of lung toxicants has been well documented (Gram, 1997). Biotransformation by means of oxygenation, reduction or dealkylation results in the formation of reactive electrophillic species such as epoxides, quinones, quinone-imines, methylen-imines and acyl radicals that react with cell biomolecules modifying them or forming covalent adducts.

Human pulmonary CYP1A1 is a prototype of an enzyme actively involved in the metabolic activation of many aromatic hydrocarbons, which generates reactive electrophillic metabolites. The metabolism of benzo(a)-pyrene in the lung constitutes a representative example of how metabolism of polycyclic aromatic hydrocarbons by CYP results in bioactivation to genotoxic metabolites which form covalent adducts with DNA. Benzo(a)pyrene is formed during incomplete combustion of organic materials, and is found in diesel exhaust, cigarette smoke and in some work-place atmospheres. The chemical is initially metabolised by CYP1A1 to form epoxides, principally benzo(a)pyrene-7,8-epoxide, which

is subsequently hydrated by epoxide hydrolase to form the corresponding 7,8-diol derivative (Fig. 5). This latter metabolite is further metabolised by CYP1A1 in the lung to benzo(a)pyrene-7,8-diol-9,10-epoxide, an extremely reactive specie that covalently binds cellular nucleophiles, among them DNA, leading to mutations and eventually altered cell growth and cancer (Burczynski and Penning, 2000; Li et al., 2001). Pulmonary CYP1B1 also participates in the activation of benzo(a)-pyrene, arylarenes, nitroarenes, and aryl amines to reactive metabolites capable of damaging cell's DNA. Interindividual differences in metabolic activation of these procarcinogens associated to genetic polymorphisms of human CYP1B1 gene have been claimed to be involved in the idiosyncratic susceptibility to lung cancer (Shimada et al., 1997; Watanabe et al., 2000; Inoue et al., 2000). Evidences exist in favour of CYP2A6 and CYP2A13 playing a significant role in human lung in the oxidative activation of tobacco-specific nitrosamines, one of the major classes of chemical carcinogens found in cigarette smoke (Jalas et al., 2003). A similar role in the bioactivation of these compounds has also been proposed for CYP2B6 (Smith et al., 2003).

Conversion of 1,1-dichloroethylene to a reactive epoxide in human lung is mediated by CYP2E1 (Dowsley et al., 1999). CYP2E1 is also involved in the bioactivation of ethyl carbamate and some nitrosamines present in tobacco smoke (Forkert et al., 2001; Smith et al., 2003). CYP2F1 is able to activate benzene, 3-methylindole and naphthalene to pneumotoxic electrophillic metabolites that covalently bound to cellular macromolecules (Lanza et al., 1999;Nichols et al., 2003). A role of CYP3A5 in the pulmonary bioactivation of nitrosamines, polycyclic aromatic hydrocarbons, 4-ipomeanol and in the generation of a reactive epoxide from aflatoxin B1 has been suggested (Czerwinski et al., 1991; Kelly et al., 1997; Piipari et al., 2000; Smith et al., 2003).

Conjugation of xenobiotics is generally associated with detoxification. However, this assessment is not fully accurate. Compounds are known that, upon conjugation with sulphate or glucuronic acid, are converted to chemically reactive metabolites. For instance, the *O*-sulphonation of a xenobiotic, because of the electron-withdrawal elicited by the sulphate group, may be heterolytic cleaved off giving rise to a strong electrophilic cation (Glatt, 2000). Studies using recombinant test systems have demonstrated demonstrate that certain pro-mutagens can be activated by specific sulphotransferases.

Glucuronyl conjugates is one of the most frequent phase II metabolites of xenobiotics. The glucuronidation is normally considered a detoxifying reaction because glucuronides usually possess less intrinsic biological and/or chemical activity than their parent aglycones. However, a number of glucuronide conjugates exist that become instable acyl derivatives when undergo nucleophillic attack by cell's protein, giving rise to chemical-protein adducts. These include two classes of glucuronides with electrophilic chemical reactivity (*N*–*O*-glucuronides of hydroxamic acids and acyl glucuronides of carboxylic acids) and several types of glucuronides that impart biological effects through non-covalent interactions (Boelsterli, 1993; Spahn-Langguth et al., 1996; Ritter, 2000). Glucuronides may thus contribute to clinically significant effects, including environmental arylamine-induced carcinogenesis, drug hypersensitivity and other toxicities associated with carboxylic acid containing drugs.

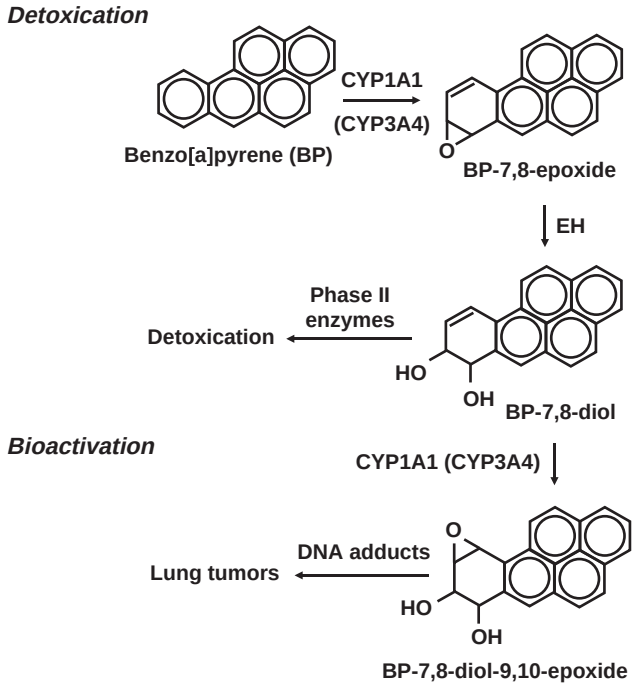

Fig. 5. Role of P450 in the bioactivation of the pre-carcinogen benzo[a]pyrene. Benzo[a]pyrene (BP) is metabolised by P450 enzymes (CYP1A1 and CYP3A4) to BP-7,8-epoxide, which is subsequently converted into BP-7,8-diol by epoxide hydrolase (EH), and into BP-7,8-diol-9,10-epoxide by CYP1A1. BP-7,8-diol-9,10-epoxide is a reactive metabolite that covalently interacts with DNA.

Cellular in vitro models for the study of metabolism and bioactivation of toxins in human lung

Knowledge about the metabolism, disposition and bioactivation of a particular compound could allow prediction of the type of lung cell damage it is likely to cause. The study of potential lung toxins would greatly benefit of having reliable experimental models able to reproduce the phenomena that may take place in the

human lung. Because of the differences in biotransformation enzyme expression across animal species, conclusions drawn from experimental animals may not be representative of what might actually occur in the human lung. The bioactivation and biological effects of a given toxin can be investigated in metabolically competent cells (i.e. cells expressing the xenobiotic biotransformation enzymes), but not on subcellular fractions (microsomes containing the enzyme activities). The spectrum of effects elicited by xenobiotics when interact with cells (bioactivation of toxins, effects on cellular metabolism, regulation of CYP expression) can only be properly visualised in a biological system fully capable of transcribing and translating genes in response to the action of toxins. Human cells isolated from pulmonary tissue meet such requirements, however, the feasibility of establishing such in vitro model is severely hampered by the difficulties in isolating and culturing functional cells from lungs (Schwarze et al., 1996). Human bronchial epithelial cells have been obtained from biopsy specimens, however, a major drawback is their limited life-span (Ramirez et al., 2004). Despite attempts have been made to immortalise human bronchial epithelium cells, the phenotype of transformed cells sensibly differ from that of normal cells (Reddel et al., 1993; Ramirez et al., 2004; Mace et al., 1997).

Xenobiotic-metabolising capability of A549 cells

In the past few years different cellular models from human pulmonary origin have been developed as experimental model to investigate the effects of xenobiotics on human lung (Yu et al., 2001). Among them, the adenocarcinoma A549 cell line, derived from type II pneumocytes, and the pulmonary adenocarcinoma NCI-H322, derived from bronchiolar Clara cells, are probably the best characterised and the most widely used (Ueng et al., 2000; Wang et al., 2002; Adissu and Schuller, 2004). In relation to the phenomenon of xenobiotic bioactivation, the weaker expression of P450 enzymes in adult Clara cells, make A549 cells the best candidate cell line for metabolism-related toxicity studies.

The morphology and cell functionality of A459 cells (i.e. surfactant synthesis, oxidative metabolism, transport properties) are consistent with that of type II pulmonary epithelial cells in vivo (Balis et al., 1984; Foster et al., 1998). In contrast to other pulmonary cell lineages, expression of P450 enzymes in A549 is not only limited to CYP1 enzyme family but also to other biotransformation enzymes. Qualitative RT-PCR analysis revealed the presence of several transcription products in A549 cells, (i.e. CYP1A1, CYP1B1, CYP2B6, CYP2Cs, CYP2E1, CYP3A5 and CYP3A7 mRNAs; Hukkanen et al., 2000).

To better characterise these cells in terms of xenobiotic metabolism gene expression, we measured by quantitative RT-PCR the mRNA content of the most relevant CYP genes and compared with that of normal lung tissue. The results (Table 3) evidenced the presence of CYP1A1, CYP1B1, CYP2B6, CYP 2Cs, CYP 3A4 and CYP3A5 in A549 cells, whereas no detectable CYP1A2, CYP2A6, CYP2A13 and CYP2E1 were found. Compared to the profile found in human lung (Table 1), CYP1B1 was the most abundantly expressed mRNA in A549 cell line, followed by CYP3A5. Although the amount of each specific P450 transcript in the cells is markedly lower than that in the lung, the profile of P450 expression show a similar trend in both systems. In agreement to these findings, the major P450 activities found in human lung microsomes could also be identified in A549 cells, although at much lower levels (Table 4). In contrast, phase II enzymes were found in the two models at comparable levels of activity (Table 4).

The responsiveness of A549 cells to enzyme inducers was also investigated. Upon exposure of cells to methylcholantrene (MC), phenobarbital (PN), rifampicin (RIF) or dexamethasone (DEX), mRNA and enzyme activity of the most relevant CYPs were determined (Figs. 6 and 7). Increased CYP1A1 and CYP1B1 mRNA levels were found after treatment with polycyclic aromatic hydrocarbon-type inducers, as previously recognised (Iwanari et al., 2002; Hukkanen et al., 2000; Dohr et al., 1997). Consistent with

Table 3. Comparison of average CYP mRNA levels in cultured A549 cells and in human lung tissue

CYP	Lung	A549 cells	Lung/A549 cells[a]
1A1	6.5±6.0	0.40±0.16	16
1A2	3.5±3.3[b]	<0.01	>350
1B1	55.0±32.2	145±48*	0.4
2A6	0.13[c]	0.09±0.03	1.4
2A13	1.53±0.40[d]	<0.01	>150
2B6	54.1±38.2	0.16±0.13*	340
2C9	14.8±11.1	1.6±1.2*	9
2E1	35.4±25.0	<0.01	>3500
3A4	8.6±4.5	0.84±0.37*	10
3A5	6.3±4.5	6.4±2.0	1.0

Specific CYP mRNAs were measured by quantitative RT-PCR and are expressed relative to β-actin mRNA content as (CYP mRNA/β-actin mRNA) $\times 10^4$ arbitrary units. Results are mean±SD of eight different lungs (unless indicated) or three different A549 cell preparations.
*$p<0.05$ respect to the corresponding mRNA content in human lung tissue.
[a]mRNA content in human lung tissue/mRNA in A549 cells.
[b]Mean of 6 lungs (see Table 1).
[c]Mean of 2 lungs (see Table 1).
[d]Mean of 3 lungs (see Table 1).

Table 4. Comparison of Phase I and II activities in cultured A549 cells and in human lung tissue

Activity	CYP	Lung	A549 cells	Lung/A549 cells[a]
Phase I				
EROD	1A/1B	<0.001	<0.001	
MROD	1A2	<0.001	<0.001	
COH	2A	30.2±26.3	0.078±0.037	387
PROD	2B6	0.351±0.416	0.001±0.001	270
6βOHT	3A	0.135±0.033	0.023±0.021*	6
ECOD	Several	1.91±0.81	0.165±0.061*	12
P450 reductase		10.3±3.0	3.6±1.1*	3
Phase II				
UGT		48.2±71.9	49.0±1.2	1
GST		45.3±12.0	31.9±18.7	1.4

Activities were measured in using appropriate substrates (see Tables 2–4) and are expressed as pmol/mg/min, except for P450 reductase and GST activities, which were expressed as nmol/mg/min. Results, are mean±SD of 7–8 lungs or three different A549 cell preparations.
*$p<0.05$ respect to the activity value in human lung tissue.
[a]Activity value in human lung tissue /activity in A549 cells.

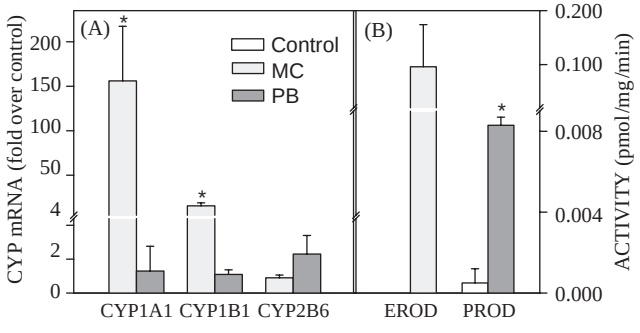

Fig. 6. Effects of model enzyme inducers on mRNA levels and activity of CYP1A, CYP1B and CYP2B enzymes in A549 cells. After 24 h of culture, A549 cells were exposed to 2 μM 3-methylcholanthrene (MC) or 1 mM phenobarbital (PB) and specific P450 mRNA content and activities were quantified 48 h later. (A) CYP1A1, CYP1B1 and CYP2B6 were quantified by RT-PCR and results are expressed as fold over corresponding CYP mRNA values in control cells. (B) 7-Ethoxyresorufin *O*-deethylase (EROD, CYP1) and 7-pentoxyresorufin *O*-dependentylase (PROD, CYP2B) activities were determined by direct incubation of cell monolayers with the appropriate substrate for 2 h and results are expressed as picomples of metabolite formed per minute and per milligrams of cellular protein. Data are mean±SD of three different cell preparations.*$p<0.05$ respect to control cells.

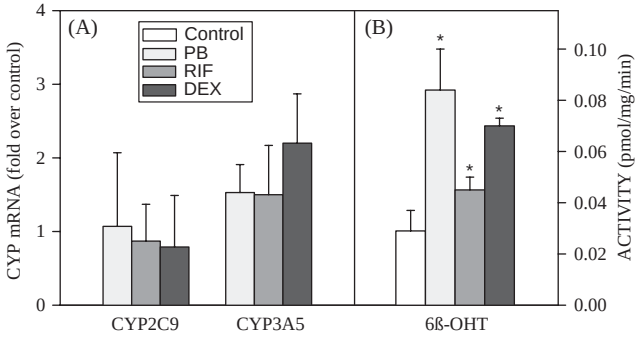

Fig. 7. Effects of model inducers on mRNA levels and activity of CYP2C, and CYP3A enzymes in A549 cells. After 24 h of culture, A549 cells were exposed to 1 mM phenobarbital (PB), 20 μM rifampicin (RIF), or 20 μM dexamethasone (DEX) and specific P450 mRNA content and activities were quantified 48 h later. (A) CYP2C9 and CYP3A5 were quantified by RT-PCR and results are expressed as fold over corresponding CYP mRNA values in control cells. (B) Testosterone 6β-hydroxylase (6β-OHT, CYP3A) activity was determined by direct incubation of cell monolayers with testosterone for 2 h and results are expressed as picomoles of metabolite formed per minute and per milligrams of cellular protein. Data are mean±SD of three different cell preparations.*$p<0.05$ respect to control cells.

these findings, we found increases in EROD activity similar to what has been previously reported (Foster et al., 1998; Hukkanen et al., 2000). The induction is selective to this type of inducer, as chemicals representative of other P450 inducers produced negligible increases in CYP1 expression. This phenomenon requires the participation of a series of proteins (AhR, Arnt, AhRR, Hsp 90, glucocorticoid receptor (GR), etc., Fig. 4) that coordinately interact with the xenobiotic to increase gene transcription. The presence of AhR, Arnt, and AhRR mRNAs has been demonstrated in A549 cells (Iwanari et al., 2002; Tsuchiya et al., 2003).

CYP1A1 and CYP1B1 may be differently regulated in human lung epithelial cells as CYP1B1 induction by polycyclic aromatic hydrocarbon showed a greater independence of AhR (Hukkanen et al., 2000). A549 cells also express GR, whereas PXR and CAR, which are needed to induce the other CYP families, were not found in A549 cells (Hukkanen et al., 2003).

Consequently, CYP3A5 is inducible by dexamethasone and other glucocorticoids through a GR-mediated mechanism, in A549 cells, whereas rifampicin and other prototypical inducers of CYP3A4 have no effects on CYP3A5 expression (Hukkanen et al., 2003). A weak inductive effect of phenobarbital on CYP3A5 has also been described (Hukkanen et al., 2000). Phenobarbital also produced a very weak induction of CYP2B6 both at mRNA and activity level (Fig. 6).

In summary, these results show that the A549 lung adenocarcinoma cell line express a limited number of the phase I and phase II enzymes involved in detoxification or bioactivation of pulmonary toxicant and respond to P450 inducers, but a much lower level that their counter partner cells in the lung.

Assessment of lung toxicants in vitro

Different human-derived in vitro models have been used in pulmonary toxicity studies (Nichols et al., 2003; Chan et al., 1999; Salmona et al.1992; McLemore et al., 1990b; Van Vleet et al., 2002). The A549 cell line is recognised a s a useful in vitro model for the assessment of damaging effects produced by inhaled chemicals, and has notably contributed to increase our knowledge about mechanisms involved in pulmonary toxicity (Cappelletti et al., 1998; Palanee et al., 2001; Sheets et al., 2004; Doyle et al., 2004; Dybdahl et al., 2004; Karlsson et al., 2005).

The A549 cell line has been extensively used in the study of human lung damage caused by paraquat (Salmona et al., 1992; Cappelletti et al., 1998). Paraquat is a well-known pneumotoxin that exerts its toxic effect by elevating intracellular levels of superoxide through a cycling redox mechanism involving reduction of paraquat cation mechanism by NADPH and transfer of electrons to oxygen (Fig. 8). Paraquat undergoes one-electron reduction by NADPH cytochrome c reductase. Under aerobic conditions the paraquat radical cation transfers its electron to dioxygen with the formation of superoxide anion, and regenerating the paraquat cation (Fig. 8). The net result of these reactions is a futile reduction–oxidation cycle in which NADPH is consumed to generate superoxide anion from molecular oxygen (Gram, 1997). Toxicity becomes evident when the defence mechanisms that protect cells from oxidant stress are overwhelmed. One of the earliest biochemical effects induced by paraquat is damage to type II pneumocytes with consequent depletion of surfactant. Mitochondrial breakage and irreversible cytoskeleton disruption have been observed in A549 adenocarcinoma cells exposed to paraquat (Wang et al., 1992; Cappelletti et al., 1994). The chemical exerts its toxic action by elevating intracellular levels of superoxide and acts as a trigger of apoptotic death in cultured cells (Cappelletti

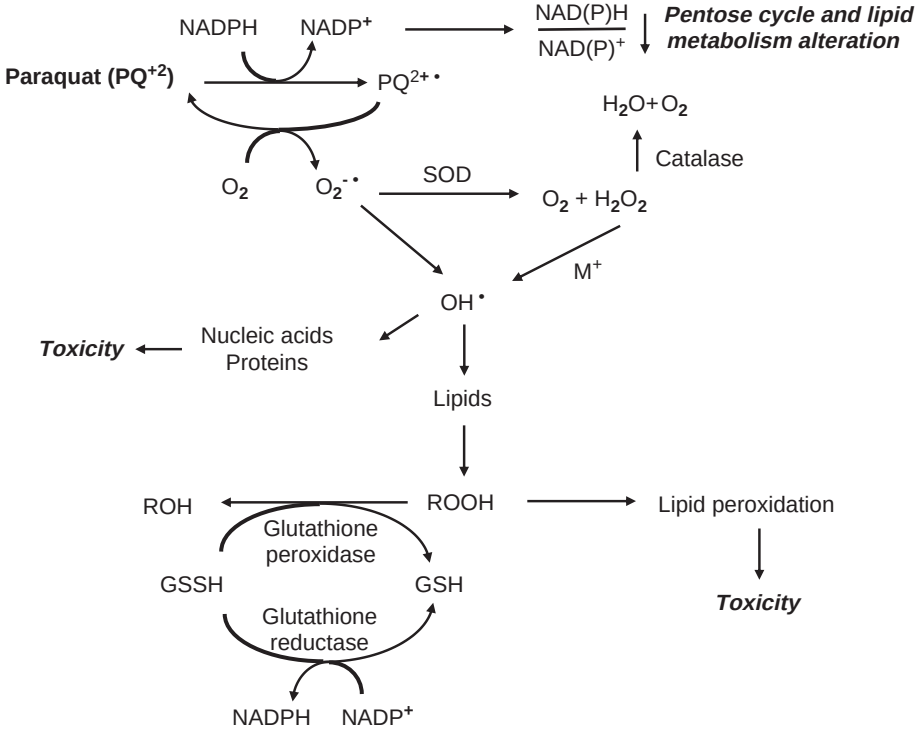

Fig. 8. Mechanism of paraquat toxicity. Paraquat cation (PQ^{2+}) is reduced to its free radical ($PQ^{+2\bullet}$) by NADPH-cytochrome P450 reductase. In the presence of O_2, $PQ^{+2\bullet}$ transfers its electron to form superoxide anion radical ($O_2^{-\bullet}$), which can be reduced to hydroxyl radical ($OH^{\bullet}$), a highly reactive oxygen species.

et al., 1998; Tomita et al., 2001). Antioxidant agents such as ascorbic acid and *N*-acetyl-cysteine have been proved effective in reducing paraquat-induced apoptosis in A549 cells.

1,3-Butadiene, a priority hazardous pollutant, constitutes another excellent example of lung toxicity study in A549 cells. P450 enzymes activate this hydrocarbon to, at least, two genotoxic metabolites in rodents, whereas indirect evidences suggested that humans may be at a decreased risk (Bond and Medinsky, 2001). Once released into the atmosphere, sunlight triggers reactions with nitrogen oxides converting 1,3-butadiene into several photoproducts. Clear evidences of cytotoxicity as well inflammatory gene expression were observed when A549 cells were exposed to those photodegradation products, whereas no adverse effects were produced by the parent hydrocarbon (Doyle et al., 2004).

Recent studies have identified an indirect genotoxicity pathway involving inflammation as one of the mechanisms underlying the carcinogenic effects of air pollution or diesel exhaust particles. Increases in the mRNA levels of pro-inflammatory cytokines and higher levels of DNA strand breaks could be observed in A549 cultures exposed to these mixtures (Dybdahl et al., 2004).

The list of environmental and occupational pulmonary toxicants which deleterious effects that have been investigated in cultured A549 cells also includes a large number of compounds and complex mixtures such as benzo(a)pyrene (Biswal et al., 2003), benzene (Sheets et al., 2004), aflatoxin B1 (Palanee et al., 2001), isoprene (Doyle et al., 2004), fluoride (Refsnes et al., 2003), tetrachlorodibenzo-*p*-dioxin (Martínez et al., 2002), naphtoquinones (Watanabe and Forman, 2003), asbestiform minerals (Gazzano et al., 2005), polyvinyl chloride particles (Xu et al., 2003), metals from combustion-derived particulate matter (Riley et al., 2005; McNeilly et al., 2004), particulate matter derived from the combustion of fossil fuels (Calcabrini et al., 2004; Karlsson et al., 2005), or cigarette smoke (Ritter et al., 2004).

Most of studies show that A549 cells are appropriate to investigate the mechanism of *active* toxins, but compounds that have to be biotransformed prior to elicit toxicity less accurately predicted with these cells. This is to be expected, in particular for those chemicals that are activated by CYP1A2, CYP2A13, and CYP2E1, enzymes not expressed in A549 cells.

The needs

As described above, the Phase I xenobiotic biotransformation capacity of A549 cells does not satisfactory reflect that of the human lung. On the contrary, Phase II activity levels found in A549 cells are in the same range of the lung tissue. Consequently, using A549 in vitro for assessing compounds with potential lung toxicity could be misleading in the case of compounds that are bioactivated by enzymes not properly expressed in those cells. This questions the suitability of A549 cells to reproduce the entire xenobiotic metabolism and bioactivation processes that is likely to occur in the human lung, and limits the relevance of the conclusions drawn from this in vitro model. Proper appraisal of toxicity caused by bioactivated metabolites formed from parent compound requires the usage of metabolically competent cells capable of generating such metabolites in situ. Since A549 cells (and the other existing human cell lines) do not fulfil this requirement, a cellular model expressing the spectrum of xenobiotic-metabolising enzymes characteristic of human lung is needed.

Three different strategies could be envisaged to confer cells xenobiotic metabolism capabilities. A simple approach could consist in transfecting A549 cells with the desired CYP genes via a vector, which contains a strong transcription promoter and integrates into DNA. Upon cloning, a new cell line expressing the transgene and an antibiotic resistance gene can be obtained. This technique, however, is limited to 1 or 2 genes whose expression level cannot be experimentally controlled.

The reason why A459 cells do not express the desired CYP genes is likely to be due to the lack or one or more transcription factors. The role of transcription factors in the expression of P450 enzymes and how they control CYP expression is reasonably well known in other CYP expressing cells (hepatocytes). By the use of expression vectors encoding key hepatic transcription factors (HNF3-γ, C/EBPs) it has been possible to re-express several CYPs, notably CYP3A4, in hepatoma cell lines (Jover et al., 2001; Rodriguez-Antona et al., 2003). Transient transfection studies have also shown that both C/EBPα and C/EBPδ transactivated the promoter of CYP2B1, the dominant P450 enzyme in rat lung, in lung epithelial cell lines (A549 and NCI-H441) (Cassel et al., 2000). This study also demonstrated the role for both C/EBPs in pulmonary expression for CYP2B1 and suggested this is a key transcription factor for the expression of P450 enzymes in the lung. Making use of vectors encoding for C/EBP factors (and other potential key factors) to transfect A549 cells, it should be possible to restore the capability of such cells to display the adult CYP phenotype.

Finally, adenoviral-derived expression vectors could be use to transiently transfect A549 cells with a combination of the most relevant human pulmonary CYPs. This experimental approach has proven to work in other cell types and allow a simultaneous and fine-tuning expression of several transgenes, without affecting the expression of other housekeeping cell's genes (Castell et al., 1997).

These strategies could contribute to achieve the ultimate goal i.e. to create a cell-based experimental model capable of identifying both *active* as well metabolism-dependent lung toxins.

Acknowledgements

Financial support was received from Forschungsgesellschaft Rauchen und Gesundheit mbH, Berlin, The Spanish Ministry of Health (Project FEDER Ref. SAF2003-09353, and Foundation ALIVE.

The technical assisstance of Ms. Gabriela Perez and Ms. Nuria Jiménez is deeply acknowledged.

References

Adissu HA, Schuller HM. Antagonistic growth regulation of cell lines derived from human lung adenocarcinomas of Clara cell and alveolar type II cell lineage: implications for chemoprevention. Int J Oncol 2004;24:1467–72.

Anttila S, Hietanen E, Vainio H, et al. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P4501A in lung cancer patients. Int J Cancer 1991;47:681–5.

Anttila S, Hukkanen J, Hakkola J, et al. Expression and localization of CYP3A4 and CYP3A5 in human lung. Am J Respir Cell Mol Biol 1997;16:242–9.

Baes M, Gulick T, Choi HS, et al. A new orphan member of the nuclear hormone receptor superfamily that interacts with a subset of retinoic acid response elements. Mol Cell Biol 1994;14:1544–52.

Balis JU, Bumgarner SD, Paciga JE, et al. Synthesis of lung surfactant-associated glycoproteins by A549 cells: description of an in vitro model for human type II cell dysfunction. Exp Lung Res 1984;6:197–213.

Bartsch H, Castegnaro M, Rojas M, et al. Expression of pulmonary cytochrome P4501A1 and carcinogen DNA adduct formation in high risk subjects for tobacco-related lung cancer. Toxicol Lett 1992;64–65:477–83.

Batt AM, Magdalou J, Vicent-Viry M, et al. Drug metabolising enzymes related to laboratory medicine: cytochromes P-450 and UDP-glucuronosyltransferases. Clin Chim Acta 1994;226:171–90.

Bertilsson G, Berkenstam A, Blomquist P. Functionally conserved xenobiotic responsive enhancer in cytochrome P450 3A7. Biochem Biophys Res Commun 2001;280: 139–44.

Biswal S, Maxwell T, Rangasamy T, et al. Modulation of benzo[a]pyrene-induced p53 DNA activity by acrolein. Carcinogenesis 2003;24:1401–6.

Boelsterli UA. Specific targets of covalent drug-protein interactions in hepatocytes and their toxicological significance in drug-induced liver injury. Drug Metab Rev 1993; 25:395–451.

Bond JA. Metabolism of xenobiotics by the respiratory tract. In: Gardner DE, editor. Toxicology of the lung. New York: Raven Press Ltd.; 1993. p. 187–215 [Chapter 7].

Bond JA, Medinsky MA. Insights into the toxicokinetics and toxicodynamics of 1,3-butadiene. Chem Biol Interact 2001;135–136:599–614.

Boyd MR. Metabolic activation and lung toxicity: a basis for cell-selective pulmonary damage by foreign chemicals. Environ Health Perspect 1984;55:47–51.

Burczynski ME, Penning TM. Genotoxic polycyclic aromatic hydrocarbon *ortho*-quinones generated by aldo-keto reductases induce CYP1A1 via nuclear translocation of the aryl hydrocarbon receptor. Cancer Res 2000;60:908–15.

Calcabrini A, Meschini S, Marra M, et al. Fine environmental particulate engenders alterations in human lung epithelial A549 cells. Environ Res 2004;95:82–91.

Cappelletti G, Incani C, Maci R. Paraquat induces irreversible actin cytoskeleton disruption in cultured human lung cells. Cell Biol Toxicol 1994;10:255–63.

Cappelletti G, Maggioni MG, Maci R. Apoptosis in human lung epithelial cells: triggering by paraquat and modulation by antioxidants. Cell Biol Int 1998;22:671–8.

Cassel TN, Gustafsson JA, Nord M. CYP2B1 is regulated by C/EBPα and C/EBPδ in lung epithelial cells. Mol Cell Biol Res Commun 2000;3:42–7.

Cassidy MK, Houston JB. In vivo capacity of hepatic and extrahepatic enzymes to conjugate phenol. Drug Metab Dispos 1984;12:619–24.

Castell JV, Hernandez D, Gomez-Foix AM, et al. Adenovirus-mediated gene transfer into human hepatocytes: analysis of the biochemical functionality of transduced cells. Gene Ther 1997;4:455–64.

Chan KW, Lee H, Wang HJ, et al. Differential response to benzo(A)pyrene in human lung adenocarcinoma cell lines: the absence of aryl hydrocarbon receptor activation. Life Sci 1999;65:1339–49.

Commandeur JNM, Stijntjes GL, Vermelulen NPE. Enzymes and transport systems involved in the formation and disposition of glutathione *S*-conjugates. Pharmacol Rev 1995;47:271–330.

Crawford EL, Weaver DA, DeMuth JP, et al. Measurement of cytochrome P450 2A6 and 2E1 gene expression in primary human bronchial epithelial cells. Carcinogenesis 1998;19: 1867–71.

Czerwinski M, McLemore TL, Gelboin HV, et al. Quantification of CYP2B7, CYP4B1, and CYPOR messenger RNAs in normal human lung and lung tumours. Cancer Res 1994; 54:1085–91.

Czerwinski M, McLemore TL, Philpot RM, et al. Metabolic activation of 4-ipomeanol by complementary DNA-expressed human cytochromes P-450: evidence for species-specific metabolism. Cancer Res 1991;51:4636–8.

Ding X, Kaminsky LS. Human extrahepatic cytochrome P450: function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. Annu Rev Pharmacol Toxicol 2003;43:149–73.

Dohr O, Sinning R, Vogel C, et al. Effect of transforming growth factor-β_1 on expression of aryl hydrocarbon receptor and genes of Ah gene battery: clues for independent down-regulation in A549 cells. Mol Pharmacol 1997; 51:703–10.

Donato MT, Castell JV, Gómez-Lechón MJ. Characterization of drug metabolizing activities in pig hepatocytes for use in

bioartificial liver devices: comparison with other hepatic cellular models. J Hepatol 1999;31:542–9.

Dowsley TF, Reid K, Petsikas D, et al. Cytochrome P-450-dependent bioactivation of 1,1-dichloroethylene to a reactive epoxide in human lung and liver microsomes. J Pharmacol Exp Ther 1999;289:641–8.

Doyle M, Sexton KG, Jeffries H, et al. Effects of 1,3-butadiene, isoprene, and their photochemical degradation products on human lung cells. Environ Health Perspect 2004;112:1488–95.

Dybdahl M, Risom L, Bornholdt J, et al. Inflammatory and genotoxic effects of diesel particles in vitro and in vivo. Mutat Res 2004;562:119–31.

Forkert PG, Lee RP, Reid K. Involvement of CYP2E1 and carboxylesterase enzymes in vinyl carbamate metabolism in human lung microsomes. Drug Metab Dispos 2001;29: 258–63.

Foster KA, Oster CG, Mayer MM, et al. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. Exp Cell Res 1998;243:359–66.

Gazzano E, Riganti C, Tomatis M, et al. Potential toxicity of nonregulated asbestiform minerals: balangeroite from the western Alps. Part 3: depletion of antioxidant defenses. J Toxicol Environ Health 2005;68:41–9.

Gonzalez FJ, Fernandez-Salguero P. The aryl hydrocarbon receptor: studies using the AHR-null mice. Drug Metab Dispos 1998;26:1194–8.

Glatt H. Sulfotransferases in the bioactivation of xenobiotics. Chem Biol Interact 2000;129:141–70.

Gram TE. Chemically reactive intermediates and pulmonary xenobiotic toxicity. Pharmacol Rev 1997;49:297–341.

Hayashi S, Watanabe J, Nakachi K, et al. Interindividual difference in expression of human Ah receptor and related P450 genes. Carcinogenesis 1994;15:801–6.

Hecht SS. Tobacco smoke carcinogens and lung cancer. J Natl Cancer Inst 1999;91:1194–210.

Hukkanen J, Lassila A, Paivarinta K, et al. Induction and regulation of xenobiotic-metabolizing cytochrome P450s in the human A549 lung adenocarcinoma cell line. Am J Respir Cell Mol Biol 2000;22:360–6.

Hukkanen J, Pelkonen O, Hakkola J, et al. Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. Crit Rev Toxicol 2002;32: 391–411.

Hukkanen J, Vaisanen T, Lassila A, et al. Regulation of CYP3A5 by glucocorticoids and cigarette smoke in human lung-derived cells. J Pharmacol Exp Ther 2003;304:745–52.

Inoue K, Asao T, Shimada T. Ethnic-related differences in the frequency distribution of genetic polymorphisms in the CYP1A1 and CYP1B1 genes in Japanese and Caucasian populations. Xenobiotica 2000;30:285–95.

Iwanari M, Nakajima M, Ryoichi K, et al. Induction of CYO1A1, CYP1A2, and CYP1B1 mRNAs by nitropolycyclic aromatic hydrocarbons in various human tissue-derived cells: chemical-, cytochrome P450 isoform-, and cell-specific differences. Arch Toxicol 2002;76:287–98.

Jalas JR, Ding X, Murphy SE. Comparative metabolism of the tobacco-specific nitrosamines 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol by rat cytochrome P450 2A3 and human cytochrome P450 2A13. Drug Metab Dispos 2003;31: 1199–202.

Jover R, Bort R, Gomez-Lechon MJ, et al. Cytochrome P450 regulation by hepatocyte nuclear factor 4 in human hepatocytes: a study using adenovirus-mediated antisense targeting. Hepatology 2001;33:668–75.

Karlsson HL, Nilsson L, Moller L. Subway particles are more genotoxic than street particles and induce oxidative stress in cultured human lung cells. Chem Res Toxicol 2005;18: 19–23.

Kehrer JP. Systemic pulmonary toxicity. In: Ballantyne B, Marrs T, Turner P, editors. General applied toxicology. Basingstoke: The Mcmillan Press Ltd.; 1993. p. 537–54 [Chapter 26].

Kelly JD, Eaton DL, Guengerich FP, et al. Aflatoxin B1 activation in human lung. Toxicol Appl Pharmacol 1997; 144:88–95.

Kim JH, Sherman ME, Curriero FC, et al. Expression of cytochromes P450 1A1 and 1B1 in human lung from smokers, non-smokers, and ex-smokers. Toxicol Appl Pharmacol 2004;199:210–9.

Lanza DL, Code E, Crespi CL, et al. Specific dehydrogenation of 3-methylindole and epoxidation of naphthalene by recombinant human CYP2F1 expressed in lymphoblastoid cells. Drug Metab Dispos 1999;27:798–803.

Li D, Firozi PF, Wang LE, et al. Sensitivity to DNA damage induced by benzo(a)pyrene diol epoxide and risk of lung cancer: a case–control analysis. Cancer Res 2001;61: 1445–50.

Mace K, Vautravers P, Granato D, et al. Development of CYP450-expressing human bronchial epithelial cell lines for in vitro pharmacotoxicologic application. In Vitro Toxicol 1997;10:85–92.

Martinez JM, Afshari CA, Bushel PR, et al. Differential toxicogenomic responses to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in malignant and nonmalignant human airway epithelial cells. Toxicol Sci 2002;69:409–23.

McLemore TL, Adelberg S, Liu MC, et al. Expression of CYP1A1 gene in patients with lung cancer: evidence for cigarette smoke-induced gene expression in normal lung tissue and for altered gene regulation in primary pulmonary carcinomas. J Natl Cancer Inst 1990a;82: 1333–9.

McLemore LT, Litterst CL, Coudert BP, et al. Metabolic activation of 4-ipomeanol in human lung, primary pulmonary carcinomas, and established human pulmonary carcinoma cell lines. J Natl Cancer Inst 1990b;82:1420–6.

McNeilly JD, Heal MR, Beverland IJ, et al. Soluble transition metals cause the pro-inflammatory effects of welding fumes in vitro. Toxicol Appl Pharmacol 2004;196:95–107.

Mimura J, Ema M, Sogawa K, et al. Identification of a novel mechanism of regulation of Ah (dioxin) receptor function. Genes Dev 1998;13:20–5.

Mori M, Tezuka F, Chiba R, et al. Atypical adenomatous hyperplasia and adenocarcinoma of the human lung: their heterology in form and analogy in immnunohistochemical characteristics. Cancer 1996;77:664–74.

Nichols WK, Mehta R, Skordos K, et al. 3-Methylindole-induced toxicity to human bronchial epithelial cell lines. Toxicol Sci 2003;71:229–36.

Nishimura M, Yaguti H, Yoshitsugu H, et al. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sentitive real-time reverse transcription PCR. Yakugaku Zasshi 2003;123:369–75.

Ohnhaus EE, Bluhm RC. Induction of the monooxygenase enzyme system in human lung. Eur J Clin Invest 1987;17:488–92.

Palanee T, Dutton MF, Chuturgoon AA. Cytotoxicity of aflatoxin B1 and its chemically synthesised epoxide derivative on the A549 human epithelioid lung cell line. Mycopathologia 2001;151:155–9.

Pearce RE, McIntyre CJ, Madan A, et al. Effects of freezing, thawing, and storing human liver microsomes on cytochrome P450 activity. Arch Biochem Biophys 1996;331:145–69.

Perez G, Tabares B, Jover R, et al. Semi-automatic quantitative RT-PCR to measure CYP induction by drugs in human hepatocytes. Toxicol In Vitro 2003;17:643–9.

Piipari R, Savela K, Nurminen T, et al. Expression of CYP1A1, CYP1B1 and CYP3A, and polycyclic aromatic hydrocarbon-DNA adduct formation in bronchoalveolar macrophages of smokers and non-smokers. Int J Cancer 2000;86:610–6.

Quattrochi LC, Guzelian PS. CYP3A regulation: from pharmacology to nuclear receptors. Drug Metab Dispos 2001;29:615–22.

Ramirez RD, Sheridan S, Girard L, et al. Immmortalization of human bronchial epithelial cells in the absence of viral oncoproteins. Cancer Res 2004;64:9027–34.

Raunio H, Hakkola J, Hukkanen J. Expression of xenobiotic-metabolizing CYPs in human pulmonary tissue. Exp Toxic Pathol 1999;51:412–7.

Raunio H, Hakkola J, Pelkonen O. Regulation of ··CYP3A genes in the human respiratory tract. Chem Biol Interact 2005;151:53–62.

REACH Proposal for a regulation of the European Parliament and of the Council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH), establishing a European Chemicals Agency and amending Directive 1999/45/EC. Brussels 29.10.2003; http://europa.eu.int/eurlex/en/com/pdf/2003/act0644en03/1.pdf

Reddel RR, Salghetti SE, Willey JC, et al. Development of tumorigenicity in simian virus 40-immortalized human bronchial epithelial cell lines. Cancer Res 1993;53:985–91.

Refsnes M, Schwarze PE, Holme JA, et al. Fluoride-induced apoptosis in human epithelial lung cells (A549 cells): role of different G protein-linked signal systems. Hum Exp Toxicol 2003;22:111–23.

Ren Q, Murphy SE, Zhen Z, et al. *O*-glucuronidation of the lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by human UDP-glucoronosyltransferases 2B7 and 1A9. Drug Metab Dispos 2000;28:1352–60.

Riley MR, Boesewetter DE, Turner RA, et al. Comparison of the sensitivity of three lung derived cell lines to metals from combustion derived particulate matter. Toxicol In Vitro 2005;19:411–9.

Ritter JK. Roles of glucuronidation and UDP-glucuronosyltransferases in xenobiotic bioactivation reactions. Chem Biol Interact 2000;129:171–93.

Ritter D, Knebel J, Aufderheide M. Comparative assessment of toxicities of mainstream smoke from commercial cigarettes. Inhal Toxicol 2004;16:691–700.

Rodriguez-Antona C, Bort R, Jover R, et al. Transcriptional regulation of human CYP3A4 basal expression by CCAAT enhancer-binding protein alpha and hepatocyte nuclear factor-3 gamma. Mol Pharmacol 2003;63:1180–9.

Saarikoski ST, Husgafvel-Pursiainen K, Hirvonen A, et al. Localization of CYP1A1 mRNA in human lung by in situ hybridisation: comparison with immunohistochemical findings. Int J Cancer 1998;77:33–9.

Salmona M, Donnini M, Perin L, et al. A novel pharmacological approach for paraquat poisoning in rat and A549 cell line using ambroxol, a lung surfactant synthesis inducer. Food Chem Toxicol 1992;30:789–94.

Schwarze PE, Johnsen NM, Samuelsen JT, et al. The use of isolated lung cells in in vitro pulmonary toxicology: studies of DNA damage, apoptosis and alteration of gene expression. Cent Eur J Public Health 1996;4(Suppl.):6–10.

Sheets PL, Yost GS, Carlson GP. Benzene metabolism in human lung cell lines BEAS-2B and A549 and cells overexpressing CYP2F1. J Biochem Mol Toxicol 2004;18:92–9.

Shimada T, Yamazaki H, Mimura M, et al. Characterization of microsomal cytochrome P450 enzymes involved in the oxidation of xenobiotic chemicals in human fetal liver and adult lungs. Drug Metab Dispos 1996;24:515–22.

Shimada T, Gillam EM, Sutter TR, et al. Oxidation of xenobiotics by recombinant human cytochrome P450 1B1. Drug Metab Dispos 1997;25:617–22.

Singhal SS, Saxena M, Ahmad H, et al. Glutathione *S*-transferases of human lung: characterization and evaluation of the protective role of the α-class isozymes against lipid peroxidation. Arch Biochem Biophys 1992;299:232–41.

Smith GBJ, Bend JR, Bedard LL, et al. Biotransformation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in peripheral human lung microsomes. Drug Metab Dispos 2003;31:1134–41.

Spahn-Langguth H, Dahms M, Hermening A. Acyl glucuronides: covalent binding an its potential relevance. Adv Exp Med Biol 1996;387:313–28.

Testa B. The metabolism of drugs and other xenobiotics. London: Academic Press; 1995.

Tomita M, Okuyama T, Ishikawa T. The role of nitric oxide in paraquat-induced cytotoxicity in the human A549 lung carcinoma cell line. Free Radic Res 2001;34:193–202.

Tsuchiya Y, Nakajima M, Itoh S, et al. Expression of aryl hydrocarbon receptor repressor in normal human tissues and inducibility by polycyclic aromatic hydrocarbons in human tumor-derived cell lines. Toxicol Sci 2003;72:253–9.

Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. Annu Rev Pharmacol Toxicol 2000;40:581–616.

Turgeon D, Carrier JS, Levesque E, et al. Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members. Endocrinology 2001;142:778–87.

Uchaipichat V, Mackenzie PI, Guo XH, et al. Human UDP-glucuronosyltransferases: isoform selectivity and kinetics of

4-methylumbelliferone and 1-naphtol glucuronidation, effects of organic solvents, and inhibition by diclofenac and probenecid. Drug Metab Dispos 2004;32:413–23.

Ueng TH, Hu SH, Chen RM, et al. Induction of cytochrome P-450 1A1 in human hepatoma HepG2 and lung adenocarcinoma NCI-H322 cells by motorcycle exhaust particulate. J Toxicol Environ Health 2000;60:101–19.

Van Vleet TR, Mace K, Coulombe RA. Comparative aflatoxin B(1) activation and cytotoxicity in human bronchial cells expressing cytochromes P450 1A2 and 3A4. Cancer Res 2002;62:105–12.

Wang HW, Chen TL, Yang PC, et al. Induction of cytochromes P450 1A1 and 1B1 in human lung adenocarcinoma CL5 cells by frying-meat emission particulate. Food Chem Toxicol 2002;40:653–61.

Wang GY, Hirai K, Shimada H. Mitochondrial breakage induced by the herbicide paraquat in cultured human lung cells. J Electron Microsc (Tokyo) 1992;41:181–4.

Wang Y, Spitz MR, schabath MB, et al. Association between glutathione *S*-transferase p1 polymorphisms and lung cancer risk in Caucasians: a case-control study. Lung Cancer 2003;40:25–32.

Watanabe J, Shimada T, Gillam EM, et al. Association of CYP1B1 genetic polymorphism with incidence to breast and lung cancer. Pharmacogenetics 2000;10:25–33.

Watanabe N, Forman HJ. Autoxidation of extracellular hydroquinones is a causative event for the cytotoxicity of menadione and DMNQ in A549-S cells. Arch Biochem Biophys 2003;411:145–57.

Waxman DJ. P450 gene induction by structurally diverse xenochemicals: central role of nuclear receptors CAR, PXR, and PPAR. Arch Biochem Biophys 1999;369:11–23.

Waxman DJ, Lapenson DP, Aoyama T, et al. Steroid hormone hydroxylase specificities of eleven cDNA-expressed human cytochrome P450s. Arch Biochem Biophys 1991;290:160–6.

Wheeler CW, Park SS, Guenthner TM. Immunochemical analysis of a cytochrome P-450IA1 homologue in human lung microsomes. Mol Pharmacol 1990;38:634–43.

Wheeler CW, Wrighton SA, Guenthner TM. Detection of human lung cytochromes P450 that are immunochemically related to cytochrome P450IIE1 and cytochrome P450IIIA. Biochem Pharmacol 1992;44:183–7.

Whitlock JP. Induction of cytochrome P4501A1. Annu Rev Pharmacol Toxicol 1999;39:103–25.

Willey JC, Coy E, frampton MW, et al. Quantitation of RT-PCR measurement of cytochromes p450 1A1, 1B1, and 2B7, microsomal epoxide hydrolase, and NADPH oxidoreductase expression in lung cells of smokers and nonsmokers. Am J Respir Cell Mol Biol 1997;17:114–24.

Xu H, Dinsdale D, Nemery B, et al. Role of residual additives in the cytotoxicity and cytokine release caused by polyvinyl chloride particles in pulmonary cell cultures. Toxicol Sci 2003;72:92–102.

Yoshimura T, Tanaka S, Horie T. Species difference and tissue distribution of uridine diphosphate-glucuronyltransferase activities towards E6080, 1-naphtol and 4-hydroxybiphenyl. J Pharmacobiodyn 1992;387:387–93.

Yost GS. Sites of metabolism: lung. In: Woolf TF, editor. Handbook of drug metabolism. New York: Marcel Dekker Inc.; 1999. p. 263–78 [Chapter 11].

Yu LJ, Matias J, Scudiero DC, et al. P450 enzyme expression patterns in the NCI human tumor cell line panel. Drug Metab Dispos 2001;29:304–12.

Zheng Z, Fang JL, Lazarus P. Glucuronidation: an important mechanism for detoxification of benzo(a)pyrene metabolites in aerodigestive tract tissue. Drug Metab Dispos 2002; 30:397–403.