cDNA has not been obtained. In the present study, four genomic DNA clones with high sequence homology to CYP2G1 were isolated from a human genomic library; one clone mapped to exons 6-9 of CYP2G1 gene and three others mapped to the 5'-end and coding sequences. PCR primers were designed according to the genomic sequence and used to amplify full length CYP2G cDNAs from human nasal mucosa. A PCR product was obtained corresponding to a cDNA of 1322 bp with over 80% identity with the coding region of rat CYP2G1. However, several stop codons were found near the 5'-end of the coding region and sequences corresponding to exon 2 were missing from this human cDNA, suggesting that it may be derived from alternative splicing of a CYP2G pseudogene. Nevertheless, an open reading frame consisting of sequences of exons 3-9 was detected. It remains to be determined whether this mRNA is translated and, if it is, whether the presumably truncated protein is functional. (Supported in part by NIH Grant DC-02640).

124 ANTAGONISM OF ARYL HYDROCARBON RECEPTOR (AHR) FUNCTION BY PD 098059.

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2 - (2' - amino - 3' - methoxyphenyl) - oxanaphthalen - 4 - one (PD 098059) is a flavonoid and a potent inhibitor of MAPK/ERK kinase (MEK). Conditions leading to constitutive activation of the MAPK pathway often suppress aryl hydrocarbon receptor (AhR) function. We examined whether PD 098059 could reverse the down-regulation of AhR function occurring in MCF10A human breast epithelial cells expressing a H-*ras* oncogene. No cytotoxicity was noted in cultures treated with PD 098059 over the range 0.1 - 20 μM. The agent was weakly cytostatic at ≥ 10 μM. Treatment of cultures with PD 098059 (≥10 μM) either at the time of addition, or 24 h prior to the addition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), totally suppressed the inductions of *CYP1A1*, *CYP1B1* and *NQO1* in wild type cells, and did not relieve the loss of inducibility characteristic of the H-*ras* transfected cells. Addition of PD 098059 to rat liver cytosol just prior to the addition of TCDD, at concentrations ≥10 μM, completely inhibited AhR transformation, as assayed *in vitro* in gel retardation assays. Immunofluorescence microscopy demonstrated a rapid translocation of the cytoplasmic AhR to the nucleus following exposure to PD 098059. Our results suggest that PD 098059 may bind to the AhR, and function as an antagonist of the receptor. Supported by NIH grant CA34469 and NIEHS center grant ES06639.

125 QUANTITATIVE RT-PCR MEASUREMENT OF CYTOCHROME P450 2A6 AND 2E1 GENE EXPRESSION IN HUMAN BRONCHIAL EPITHELIAL CELLS.

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Inhalation of nitrosamine procarcinogens in combustion products is thought to increase the risk for bronchogenic carcinoma. Nitrosamines are metabolized to carcinogenic products through the action of cyclooxygenases, including the cytochrome p450 (cyp450) isozymes 2A6 and 2E1. Because carcinogens are highly reactive with proteins as well as nucleic acids, their effects are likely to be concentrated in the cells in which they are activated. In an effort to quantify metabolic determinants of risk for bronchogenic carcinoma, we measured CYP2A6 and CYP2E1 gene expression in human bronchial epithelial cells (BEC) obtained bronchoscopically from 12 non-smokers and 8 smokers. Using modifications of our previously described multiplex competitive RT-PCR methods (Apostolakos et al. Analyt. Biochem., 1993), we determined that CYP 2A6 was expressed at quantifiable levels in BEC from 12/12 non-smokers but only 6/8 smokers. In addition, the mean level of CYP2A6 expression was significantly higher ($p < 0.05$) in non-smokers compared to smokers (690 and 240 mRNAs/10^6 β actin mRNAs respectively). Further there was significant inter-individual variation ($p < 0.05$) in CYP2A6 expression among both smokers and non-smokers. CYP2E1 was expressed in the BEC of 9/20 individuals, but in each case at levels below the limit for quantification (10 mRNAs/10^6 β-actin mRNAs). We hypothesize that phase I metabolism of nitrosamines in BEC is catalyzed primarily by CYP2A6, that inter-individual variation in risk for bronchogenic carcinoma is determined in part by CYP2A6 expression in BEC, and that cigarette smoking reduces CYP2A6 expression in BEC of some individuals.

126 INTRA-INDIVIDUAL VARIATION OF CYTOCHROME P450 FORMS IN HUMAN HEPATIC MICROSOMES: CORRELATION OF INDIVIDUAL FORMS WITH XENOBIOTIC METABOLISM.

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We and others have previously demonstrated that P-450 dependent trichloroethylene (TRI) metabolism is highest in microsomes from mice, somewhat lower in rats and substantially lower in pooled human microsomes. In this study we report on levels of 6 P450 forms (as measured by a direct ELISA) and microsomal metabolism toward TRI in relation to specific content of P450 forms in these samples. Cytochrome P450 forms were quantified in human microsomes using antibodies specific to rat P450s CYP1A, CYP2B, CYP2C6, CYP2C11, CYP2E1 and CYP3A. Protein-content for CYP1A, CYP2E1 and CYP3A were positively correlated with microsomal activities that are recognized as suitable markers for those forms. CYP1A content varied approximately 5 fold between the highest and lowest human samples tested. CYP2B, CYP2C6-reactive proteins, CYP2E1 and CYP3A demonstrated approximately 10-fold differences between the highest and lowest samples. CYP2C11-reactive microsomal protein displayed minimal differences in content among the samples tested. P450 content of specific P450 forms was not correlated to total P450. When comparing levels of specific P450 forms to TRI metabolism, CYP2E1 was strongly correlated to high TRI-Vmax and chloral hydrate formation; whereas CYP2B displayed the strongest correlation toward formation of trichloroethanol. These results indicate that humans are not uniform in their capacity to metabolize TRI, and that factors which result in increased CYP2E1 and/or CYP2B activity may increase susceptibility to TRI-induced toxicity in the human.

127 INDUCTION OF CYP2A6 AND CYP3A MRNA IN CULTURED HUMAN HEPATOCYTES BY RIFAMPIN: MEASUREMENT WITH QUANTITATIVE RT-PCR.

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Hepatic enzyme induction is often evoked as the cause for a variety of effects in animal studies, eg, hepatic hypertrophy and secondary thyroid neoplasms in rodents. In clinical practice, enzyme induction often enhances drug clearance and may result in reduced efficacy. For example, carbamazepine or rifampin treatment induces P450 3A in humans and, as a result, dramatically reduces the efficacy of midazolam or cyclosporine. Due to species differences in substrate specificities and the regulation of various drug-metabolizing enzymes, assessing enzyme induction in human tissues is a desirable goal. Since induction often occurs as a result of increased synthesis of mRNA coding for a particular enzyme, induction may be quantitated by measuring specific mRNA levels. We describe experiments using primary human hepatocytes cultured on collagen-coated plates and treated with 0, 1, 10 and 33 μM rifampin for 72 hours. Total RNA was isolated and analyzed for specific cytochrome P450 mRNAs using a simple, quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Both Cyp3A and Cyp2A6 mRNAs were induced in a dose-dependent fashion by rifampin treatment. Induction in the high-dose samples was ~10-fold for Cyp3A mRNA and ~4.5 fold for Cyp2A6 mRNA. By contrast, mRNA levels for Cyp1A1 and Cyp2E1 were not increased by rifampin treatment. This is the first report of Cyp2A6 induction by rifampin, and it demonstrates the potential of this system for examining specific enzyme induction in human liver cells.

128 DOES EXPRESSION OF CYP1A1 IN HUMAN LUNGS DEPEND ON POLYMORPHIC GSTs ?

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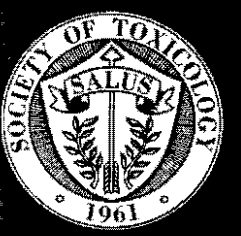
Several polynuclear aromatic compounds, which are present in cigarette smoke, are known substrates and inducers of *CYP1A1* expression. The oxidized metabolites can then be substrates of various forms of glutathione S-transferase (GST). The majority (85-90%) of lung cancer patients who are current smokers express *CYP1A1* in the lung tissue. Since only about 10% of Caucasians are considered to exhibit high inducibility of *CYP1A1*, this suggests that smokers who have inducible *CYP1A1* are at a higher risk of developing lung cancer. Roughly one half of Caucasians have a null allele, *GSTM1*0*, and consequently have no GSTM1 enzyme. One tenth of Cauca-



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshops, roundtables, and poster sessions of the 36th Annual Meeting of the Society of Toxicology, held at the Cincinnati Convention Center, Cincinnati, Ohio, March 9-13, 1997.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 371.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 395.

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