1918 HUMAN AND RAT CYTOCHROME P450 ISOFORM SELECTIVITY WITHIN A PANEL OF FLUOROMETRIC SUBSTRATES.

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The advantages of fluorescence detection in enzyme assays (*e.g.*, sensitivity, ease of use, etc) are well-known. In this study we have examined the relative catalytic selectivity of more than 25 rat or human cDNA-expressed cytochromes P450 toward a panel of 7 fluorometric substrates. The substrates examined were dibenzyl fluorescein, 7-benzyloxyquinoline (BQ), 3-cyano-7-ethoxycoumarin, 7-methoxy-4-trifluoromethylcoumarin, 3-[2-(N, N-diethyl-N-methylamino)ethyl]-7-methoxy-4-methylcoumarin (AMMC), resorufin benzyl ether, and 7-benzyloxy-4-trifluoromethylcoumarin (BFC). Within each substrate, experimental conditions were chosen based on those optimized for a particular isoform of interest (*e.g.*, CYP2D6 for AMMC). For most substrates, multiple isoforms were found to catalyzed the formation of the fluorescent product. However, among the isoforms tested, rat CYP2D2 and human CYP2D6 displayed high selectivity with AMMC and human CYP3A enzymes were selective with BQ and BFC. All enzymes could dealkylate at least one substrate with the exception of rat CYP2A1. The catalytic profiles for each substrate were distinct. These data illustrate the utility of fluorescent substrate probes for detecting catalytic activity in liver microsomes or cDNA-expressed cytochromes P450.

1919 QUANTIFYING CELLULAR EXPRESSION OF CYTOCHROME P450 1A1 (CYP1A1) IN THE PULMONARY ALVEOLUS: A COMPARISON OF INDIRECT ENZYMATIC IMMUNOHISTOCHEMISTRY (IH) AND INDIRECT IMMUNOFLUORESCENCE (IF).

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Two common immunologic detection systems, IH and IF were compared for use with morphometric assessment of CYP1A1 induction in the alveolar region of the lung. Rats were treated intraperitoneally with either a known inducer of CYP1A1; *i.e.*, 50 mg/kg β-naphthoflavone(NF) in corn oil (n=4), or corn oil alone (n=4). Lungs were perfusion-fixed with neutral buffered formalin and the left lateral lung lobe was paraffin embedded within 24 hours of collection. For IH, CYP1A1 was localized using a streptavidin-biotin, peroxidase detection system and 3-amino-9-ethylcarbazole as the chromogen. For IF, CYP1A1 was localized using an Alexa 594 conjugated goat anti-rabbit antibody and cytokeratin 8 (a marker for type II cells in the alveolus) was localized using Alexa 488 conjugated donkey anti-sheep antibody. For both techniques, the primary antibody was a rabbit polyclonal antibody which reacted with rat CYP1A1. Four representative alveolar areas were captured by digital photomicroscopy and images were analyzed using computerized morphometric analysis. The area of lung occupied by CYP1A1 positive cells was significantly increased in NF injected rats using either technique to visualize CYP1A1 containing cells. Using digital capture to minimize fading of fluorochromes, IF was significantly more sensitive than IH ($P < 0.05$). By colocalization analysis, most of the area containing CYP1A1 after induction was not located in an area also expressing cytokeratin 8. Thus, IF was able to localize the site of CYP1A1 expression in the alveolus and indicated that most of the alveolar CYP1A1 induction was not in the type II cells. Cellular morphology was better demonstrated by IH than by IF. Both IH and IF were useful in detecting an increased area of the alveolus involved in CYP1A1 expression in formalin-fixed lung after exposure to the CYP1A1 inducer, NF.

1920 EXPRESSION OF ARYL HYDROCARBON RECEPTOR, ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR, AND CYTOCHROME P4501A1 IN LUNG CANCER.

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Cytochrome P4501A1 (CYP1A1) is involved in metabolic activation of polycyclic aromatic hydrocarbons (PAH) in non-hepatic tissues. CYP1A1 inducibility has been considered to be important for individual susceptibility to lung cancer. CYP1A1 induction by PAH is mediated through ligand-dependent heterodimerization between aryl hydrocarbon receptor (AhR) and aryl hydrocarbon receptor nuclear translocator (Arnt). In the present study we examined AhR, Arnt and CYP1A1 expression in human lung tumor tissues by immunohistochemistry. AhR, Arnt and CYP1A1 were mainly present in the bronchiolar epithelium and neoplasm. The staining of AhR and CYP1A1 were located in the cytosol, nevertheless, Arnt in the nuclei of epithelial cells. AhR levels were significantly higher in adenocarcinomas and females. CYP1A1 levels were also higher in adenocarcinomas than in squamous cell carcinomas. Furthermore, CYP1A1 levels correlated with AhR levels among nonsmoking adenocarcinomas. Our data suggest that high AhR expression may contribute to high CYP1A1 expression and increased risk of developing lung adenocarcinoma in nonsmokers.

1921 THE ROLE OF CYTOCHROME P450 IN THE METABOLISM OF [^{13}C/^{14}C]STYRENE.

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Rat, mouse, and human differences occur for the metabolism of styrene. The greater mouse sensitivity to styrene is believed to be related to a higher production of metabolites that are derived from the intermediate phenylacetaldehyde (PAA) or from ring-epoxidation of styrene. In this study, the role of cytochrome P450 in the metabolism of styrene was evaluated. Wild-type (WT) mice, mice devoid of cytochrome P4502E1 (CYP2E1-null), and CYP2E1-null mice pretreated with aminobenzotriazole (ABT) were exposed for 6 hr to a 250-ppm mixture of [$^{13}C_8$]styrene and [^{14}C]styrene *via* nose-only inhalation. Urine, feces, and expired volatiles were collected for up to 24 hr, and mice were sacrificed for the collection of blood and tissues. A greater excretion of total urinary metabolites and total expired [^{14}C]styrene-equivalents was apparent for null mice compared to WT mice. WT mice, CYP2E1-null mice, and ABT-CYP2E1-null mice excreted metabolites derived from conjugation of styrene oxide with glutathione (~40% of excreted metabolites). WT mice excreted a slightly higher percentage of metabolites derived from hydrolysis of styrene oxide (28%) and a lower percentage of metabolites derived from PAA (17%) compared with null mice (16 and 25%, respectively). These data suggest that CYP2E1 may not be a major isozyme involved in the in vivo metabolism of styrene to styrene oxide in mice.

1922 SITE DIRECTED MUTAGENESIS OF CYTOCHROME P450 2F3: AN EXCLUSIVE DEHYDROGENASE OF THE PNEUMOTOXIN 3-METHYLINDOLE.

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3-Methyindole is a potent pneumotoxin that requires P450-mediated dehydrogenation to elicit its toxicity. Cytochrome P450 2F3 is a pulmonary selective isozyme that has been shown to dehydrogenate 3-methylindole exclusively. Dehydrogenation is a somewhat newly discovered and unique catalytic pathway for P450 enzymes. However, these reactions are becoming increasingly important to the study of reactive intermediate formation and drug metabolism. Cytochrome P450 2F3-mediated oxidation of 3-methylindole to the dehydrogenated intermediate represents an ideal environment in which to study the biochemical and catalytic requirements of this unique P450 pathway at the enzyme level. Four site-directed, double mutants of cytochrome P450 2F3 have been successfully generated. Mutations were introduced using a commercially available PCR-based mutagenesis kit. Target sites for mutation were chosen based on known substrate recognition and contact regions as determined by both cytochrome P450 2 family sequence homology and prior mutagenesis studies. Correct incorporation of the intended mutation was confirmed by full-length sequence analysis for each of the four mutants. Protein expression studies were conducted in *E. coli*. Three of the four mutants produced active cytochrome P450 as determined by spectral studies of the enzyme within a bacterial membrane preparation. Preliminary studies of the mutants' catalytic properties suggest that each has retained dehydrogenase activity for the known 2F3 substrate 3-methylindole. However, one mutant produced a previously uncharacterized metabolite of 3-methylindole, which may arise *via* oxygen incorporation as opposed to dehydrogenation. Studies are ongoing to fully characterize the mutant enzymes' catalytic properties for 3-methylindole in addition to other known substrates for the 2F family.

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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