136 METABOLISM OF 1,2,4-BENZENETRIOL BY HUMAN MYELOPEROXIDASE: IMPLICATIONS IN BENZENE-INDUCED MYELOTOXICITY. V V Subrahmanyam, P Kolachana and M T Smith, School of Public Health, University of California, Berkeley, CA.

1,2,4-Benzenetriol is a minor urinary metabolite of benzene, a known human myelotoxin and leukemogen. Previously, it has been thought benzenetriol is mainly autooxidized in aqueous solutions to reactive quinones generating hydrogen peroxide as a by-product. Here, we report that benzenetriol oxidation is greatly stimulated by human myeloperoxidase if the reaction is performed at pH 7.4. This suggests that hydrogen peroxide generated during benzenetriol autooxidation could enhance the myeloperoxidase-mediated metabolism of benzene. In contrast, under acidic conditions (pH 5) benzenetriol metabolism by myeloperoxidase required the addition of hydrogen peroxide. In addition, we found that benzenetriol did not autooxidize at appreciable rates in acidic conditions. Initial spectrophotometric studies indicate that benzenetriol is mainly oxidized to quinone derivative(s) having an absorbance maximum between 480 and 500 nm. HPLC coupled with electrochemical detection in the reductive mode resulted in the identification of two quinone metabolites of benzenetriol. We suggest that these quinone metabolites of benzenetriol may contribute to the myelotoxic effects of benzene.
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137 METABOLISM OF BIS(2-METHOXYETHYL)ETHER BY RAT AND HUMAN LIVER MICROSOMES. M A Tirmenstein. Cellular Toxicology Section, ETB, DBBS, NIOSH, CDC. Taft Laboratories, Cincinnati, OH. Sponsor: M A Toraason.

Bis(2-methoxyethyl)ether (diglyme) is a member of the glycol ether class of chemicals. These compounds are widely used as industrial solvents. Previous studies have shown that diglyme is metabolized in vivo by cleavage of the central ether linkage to 2-methoxyethanol (2-ME). 2-ME exposure has been associated with teratogenesis, immunotoxicity, testicular atrophy and hemopoietic toxicity in experimental animals. The present study was designed to assess the effects of various cytochrome P-450 inducers on diglyme metabolism and to compare human and rat liver metabolism of diglyme. Rat liver microsomes exhibited NADPH-dependent metabolism of diglyme to 2-ME, 2-(2-methoxyethoxy)ethanol and an unidentified metabolite. These products were also detected in incubations with NADPH and human liver microsomes although there were quantitative differences in the amounts of each metabolite formed between human and rat microsomes. Induction of rat microsomal enzymes with phenobarbital or ethanol increased the conversion of diglyme to methoxyethanol by a factor of 5.6 and 4.2 fold respectively over controls. However, β-naphthoflavone induction did not significantly increase diglyme cleavage to 2-ME. Pretreatment of rats with 0.6% diglyme in drinking water for 4 days significantly increased cytochrome P-450 and P-450 reductase levels in liver microsomes. In addition, there was an increase in P-450 associated enzyme activities (ethoxycoumarin deethylase and aniline hydroxylase activities) following the diglyme pretreatment. Microsomes from rats pretreated with diglyme exhibited a 2.4 fold increase in rates of diglyme cleavage as compared to controls. These results indicate that induction of rats with specific P-450 inducers can increase the formation of 2-ME from diglyme by rat liver microsomes. Also, diglyme pretreatment induced cytochrome P-450 levels in rat liver microsomes and apparently increased the activity of those P-450 isozymes associated with the cleavage of diglyme to 2-ME.

138 ROLE OF THE FLAVIN-CONTAINING MONOOXYGENASE AND CYTOCHROME P450 ENZYMES IN THE OXIDATION OF THE PSYCHOACTIVE DRUG THIORIDAZINE. BL Blake, RL Rose, K Venkatesh, PE Levi, and E Hodgson. Department of Toxicology, North Carolina State University, Raleigh, NC.

Oxidation of the antipsychotic drug thioridazine is the primary pathway whereby both pharmacologically active and toxic metabolites are formed. Thioridazine-S-oxides are known to contribute to the activity of the drug as well as to the side effects experienced by many patients. The significance of the formation of other oxidation products such as N-oxides and phenolic metabolites, as well as the demethylated product is unknown. Both cytochrome P450 and the microsomal flavin-containing monooxygenase (FMO) enzymes have been implicated in these metabolic pathways as a result of their oxidative characteristics. The microsomal metabolism of thioridazine was examined in mouse liver tissues. Using inhibitors of cytochrome P450 and FMO, as well as the purified enzymes, the respective contribution of each enzyme was analyzed under varying conditions. Cytochrome P450 oxidized the compound at a number of sites, with the main product being thioridazine-2-sulfoxide. Northioridazine, the N-demethylated product, was also formed by this enzyme. The FMO enzyme produced mainly thioridazine-N-oxide, and to a lesser extent, thioridazine-2-sulfoxide and at least one other, as yet unidentified product.

139 STEREOSELECTIVE SULFOXIDATION BY HUMAN FLAVIN-CONTAINING MONOOXYGENASES:CATALYTIC DIVERSITY BETWEEN HEPATIC, RENAL AND FETAL FORMS. A J M Sadeque and A E Rettie. Department of Medicinal Chemistry, University of Washington, Seattle, WA. Sponsor: S D Nelson

Purified microsomal flavin-containing monooxygenases (FMO) from rabbit lung and mini-pig liver, forms which represent distinct gene sub-families, previously have been shown to catalyse the oxidation of methyl *p*-tolyl sulfide to the corresponding *R*-(+)-sulfoxide with high (>90%) stereoselectivity. In this study we have evaluated the stereoselectivity of the NADPH-dependent sulfoxidation of methyl *p*-tolyl sulfide catalysed by detergent-solubilised microsomes from adult and fetal human tissues. Microsomal preparations were solubilised in the presence of glycerol and Lubrol PX, and incubated at pH 8.5 with 100 μM substrate. Under these conditions only sulfoxide metabolites were detected. Analysis was performed by silica and chiral-phase HPLC. Three fetal liver samples (90-150 days) formed sulfoxide with 93-94% *R*-(+) stereochemistry. Adult human kidney also exhibited high [98% *R*-(+)] stereoselectivity as did liver preparations from mini-pigs, rats, rabbits, mice and hamsters [91 $\pm$ 3% *R*-(+)]. However, in contrast to all previously examined enzyme sources, adult human liver preparations obtained from twelve individuals, including the kidney donor, showed little stereopreference [50-69% *R*-(+)] for the reaction. Methimazole (1mM) decreased the rate of formation of sulfoxide catalysed by adult liver preparations by 37%, while stereochemistry was unchanged. In addition, heat treatment of adult human liver preparations (43^{o}C for 3 minutes in the absence of NADPH) decreased sulfoxide formation to 18% of control values while inclusion of NADPH during preincubation at 43^{o}C restored activity to 70% of control values. Similar findings were obtained with fetal liver and adult kidney. These data suggest that sulfoxide formation in each of the detergent-solubilised human microsomal preparations can be attributed to FMO and that significant catalytic differences exist between hepatic, renal and fetal form(s) of the enzyme in humans. This work was supported by NIH grant GM 43511.

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