179, and the other with a positive ion of molecular weight 209, which were identified as 5-allyl-1-methoxy-2, 3-dihydroxybenzene (the catechol metabolite) and 1'-hydroxymyristicin, respectively. Samples from the rat liver microsomal incubations were further analyzed by GC/MS to confirm the identity of the these two metabolites. UV absorption data were used to compare relative amounts of metabolites between samples. In all cases the catechol metabolite was the principal product of CYP activity, and in both species its formation was more rapid using microsomes from PB-induced livers. After 1 hour at 37° C with 1 mg/ml microsomal protein, the catechol metabolite accounted for 89.9 ± 4.5 % of the metabolites formed by PB-induced rat microsomes, and 84.1 ± 3.1 % of metabolites formed by pooled rat microsomes. Under these same conditions with human liver microsomes, the catechol metabolite was 73.8 ± 0.7 % of the total metabolites from PB-induced liver microsomes and 83.0 ± 2.5 % of the total from pooled human liver microsomes. PB induction of 1'-hydroxymyristicn formation was more pronounced in human microsomes than in rat microsomes. Preliminary studies incubating myristicin with rat and human primary hepatocytes attached to collagen-coated plates produced no evidence for formation of either of these two metabolites by intact cells. (*Supported by NIEHS Contract No. N01-ES-95437*).

1552 EXTRAHEPATIC METABOLISM OF BIOCHANIN A AND FORMONONETIN AND METABOLITE INHIBITION OF CYTOCHROME P450 1B1.

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Biochanin A (BA) and formononetin (FOR), are the 4′-O-methyl derivatives of the more potent phytoestrogens genistein (GEN) and daidzein (DA), respectively, and are the predominant isoflavones in red clover and chick peas. Previously, we demonstrated that human liver microsomes converted BA and FOR to GEN and DA. Since epidemiologic studies for breast cancer risk associate exposure to endogeneous estrogens with increased risk and soy diets high in DA and GEN with reduced risk, we investigated the extrahepatic metabolism of BA and FOR. Metabolic activation of estradiol (E2) to estrogen catechols is a prerequisite for its genotoxic activity with 4-hydroxylation leading to carcinogenicity and 2-hydroxylation associated with anticarcinogenicity. In humans, cytochrome P450 1B1 (1B1) is an extrahepatic E2 4-hydroxylase that activates procarcinogens, and elevated levels have been associated with estrogen carcinogenesis. We demonstrated 1B1-catalyzed O-demethylation of BA and FOR producing GEN and DA, which feedback to inhibit 1B1. Recombinant human 1B1 was incubated with BA or FOR in reactions containing NADPH regenerating systems and products were analyzed by HPLC with UV and coulometric detection. FOR was converted to DA and several hydroxylated metabolites and BA was converted to GEN and a different set of hydroxylated metabolites. DA and GEN were identified by their retention times and electrochemical oxidation profiles and confirmed by LC-MS. Inhibition of 1B1 7-ethoxyresorufin O-deethylase (EROD) activity by DA and GEN was determined using a 96-well plate assay. Inhibition of 1B1 EROD activity by GEN was primarily noncompetitive (Ki 1.9 μM). DA exhibited mixed, but predominantly noncompetitive inhibition of 1B1 EROD activity (Ki 3.7 μM). The data suggest that BA or FOR may exert anticarcinogenic effects directly by acting as competitive substrates for 1B1 or indirectly through their metabolites DA and GEN, which are both 1B1 inhibitors and more potent phytoestrogens than their methylated precusors.

1553 P450 INHIBITION BY METHYLENEDIOXYPHENYL COMPOUNDS PRESENT IN GOLDENSEAL.

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With the widespread use of nutritional supplements, pharmacokinetic interactions between botanicals and prescription or OTC drugs are of increasing concern. Goldenseal (Hydrastis canadensis) is a popular immunostimulant botanical that is widely available, either alone or in combination, with Echinacea. Earlier studies indicated that all three of the major methylenedioxyphenyl (MDP) compounds contained in goldenseal are able to inhibit *in vitro* cytochrome P450 catalyzed diclofenac-4'-hydroxylation (CYP2C9), bufuralol-1'-hydroxylation (CYP2D6), and testosterone-6β-hydroxylation (CYP3A4) activities, in human liver microsomes. MDP compounds are known for their ability to inhibit *via* the formation of metabolic-intermediate (MI) complexes with cytochrome P450. Therefore, a study was initiated to investigate the degree to which the inhibition of human cytochrome P450s by MDP compounds of goldenseal correlated with the formation of such complexes. Despite inhibiting CYP2C9, CYP2D6 and CYP3A4 activities, neither berberine nor hydrastinine were able to form MI complexes in human liver microsomes. Both the (+) and (-) isomers of hydrastine formed an MI complex. To delineate the CYP(s) responsible, MI complex formation was further investigated using supersomes (Gentest) containing a single CYP isoform. (+)-Hydrastine formed complexes with CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 but not CYP2B6. (-)-Hydrastine formed complexes only with CYP1A2, CYP2C9, CYP2D6, and CYP3A4. Co-expression of cytochrome b5 with CYP3A4 and CYP2C9 in the supersomes significantly enhanced the rate of MI complex formation. Therefore, inhibition of multiple human P450s by MDP components present in goldenseal may arise from the quasi-irreversible inhibition by MI complex formation, but other mechanisms also contribute to the inhibition.

1554 HYDROXYL RADICAL FORMATION BY HETEROLOGOUSLY EXPRESSED MICROSOMAL ENZYMES.

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It has been shown that the hydroxylation of terephthalate (TPT) to the highly fluorescent product 2-hydroxyterephthalate (2-OH TPT), is the result of reaction with hydroxyl radicals (●OH). Using a new method (Mishin and Thomas, 2002), we observed the formation of 2-OH TPT from TPT by cDNA-expressed: NADPH-cytochrome P450 oxidoreductase (OR) coexpressed with cytochrome b5 and rat cytochrome P450 enzymes (CYPs) coexpressed with OR and cytochrome b5. The formation of 2-OH TPT totally depended on the presence of NADPH and Fe/EDTA (or Fe/citrate) complexes. The 2-OH TPT generation was profoundly inhibited by DMSO a ●OH scavenger, catalase and glutathione peroxidase (+GSH), but superoxide dismutase did not have a significant effect. Several water-soluble substrates, specific for different CYP enzymes, (zoxazolamine, benzphetamine, chlorzoxazone, midazolam and buprenorphine) were without inhibitory effect on the formation of 2-OH TPT from TPT. A comparison of the relative rates of 2-OH TPT generation (nmoles 2-OH TPT/nmole P450/min) revealed CYP2A2 (23.4) and CYP2C6 (23.4) to have the highest rates and CYP2C12 (7.5) and CYP3A2 (8.1) to have the lowest rates. The other 12 CYPs-containing preparations, including CYP2E1, had similar rates that were intermediate. There was no correlation between OR levels in these expressed microsomal preparations and formation of 2-OH TPT even though OR alone (+NADPH) is sufficient for modest production of 2-OH TPT. We suggest that the generation of reactive hydroxyl radicals by microsomal enzymes proceeds *via* metal-catalysed Haber-Weiss reactions and is controlled by the rate of hydrogen peroxide formation by cytochrome P450 enzymes and redox cycling of iron ions.

1555 LUMINESCENT CYTOCHROME P450 ASSAYS THAT UTILIZE D-LUCIFERIN DERIVATIVES AS PROBE SUBSTRATES.

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Cytochrome p450s (cyp450) are the main catalyst of the oxidative metabolism of drugs and other xenobiotics. Understanding how potential new drugs interact as substrates or inhibitors of cyp450s is an essential component of drug development. To facilitate the rapid screening of multiple compounds we tested derivatives of (4S)-4, 5-dihydro-2-(6-hydroxybenzothiazolyl)-4-thiazolecarboxylic acid (D-luciferin) as probe cyp450 substrates. The D-luciferin derivatives were not substrates for luciferase in light-generating reactions but were metabolized by cyp450s to D-luciferin, which in turn reacted with firefly luciferase to produce light. The reactions were formulated using a homogenous luciferase mixture that was added directly to a conventional cyp450 reaction with D-luciferin derivatives as substrates to produce a glow-style luminescent signal that was stable for several hours. The amount of D-luciferin produced by a cyp450 was proportional to the light output of the luciferase reaction. Light was therefore used as a measure of cyp450 activity. In accord with this scheme 6′-deoxyluciferin was hydroxylated by human cyp2C9 to form D-luciferin that was readily detected by bioluminescence. 6′-deoxyluciferin was highly selective for cyp2C9 over other cyp450s. Other reactions included the dealkylation of luciferin 6′ alkyl and substituted alkyl ethers to form D-luciferin by human cyp1A1, 1A2, 2C8, 2C9 and 3A4 with varying degrees of cyp450 isoform selectivity. The luminescent cyp450 assays detected known cyp450 inhibitors with IC50s similar to those reported using conventional substrates. The assays displayed exquisite sensitivity detecting the activity of as little as 0.01 pmoles recombinant cyp450 against a minimal or immeasurable background signal and were easily configured in multi-well plate formats. We conclude that certain D-luciferin derivatives can be used as probe cyp450 substrates in simple and robust luminescent assay formats for rapid screening of multiple compounds against cyp450 activities.

1555a RAT PULMONARY CYP1A1 INDUCTION IS INHIBITED BY RESPIRABLE COAL DUST EXPOSURE.

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Cytochrome P450 1A1 (CYP1A1) metabolizes polycyclic aromatic hydrocarbons in cigarette smoke to reactive intermediates that can initiate lung cancer. We hypothesized that coal dust (CD) exposure might modify pulmonary carcinogenesis

by altering pulmonary CYP1A1 induction. To test this hypothesis, we examined the ability of respirable CD particles (<5 microns) to inhibit pulmonary CYP1A1 induction. Male, Sprague-Dawley rats (220-270g) were intratracheally exposed to 0, 2.5, 10, 20, 40 mg coal dust/rat or vehicle (saline). After 11 days, rats were injected intraperitoneally (IP) with the CYP1A1 inducer β-naphthoflavone (BNF: 50mg/kg IP). Three days later, rats were sacrificed and CYP1A1 activity in the lungs was measured as 7-ethoxyresorufin-O-deethylase (EROD) activity. CYP1A1 protein was determined by Western blot using polyclonal rabbit anti-rat CYP1A1 antibodies. Pulmonary inflammation was assessed by determining bronchoalveolar lavage polymorphonuclear (PMN) cell counts, alveolar macrophage (AM) chemiluminescence (CL), and nitric oxide (NO)-dependent AM chemiluminescence. EROD activity was suppressed by CD exposure in a dose-dependent fashion (R2= 0.932, p=0.008). Western blot showed a significant reduction of CYP1A1 protein in rats treated with 40 mg CD and BNF when compared with rats treated with BNF alone ($p<0.05$). CD exposed rats had a dose-dependent increase of PMN (R2=0.974, p= 0.002). AM count was significantly higher in all rats exposed to CD and BNF compared with rats treated with BNF alone. NO-dependent CL was also significantly increased in rats treated with 40 mg CD and BNF compared to rats treated with BNF alone (p= 0.004). These results suggest that coal dust exposure inhibits induction of CYP1A1 activity by BNF and enhances pulmonary inflammation, in a dose-dependent manner.

1556 METABOLITE OF PNU-142721 FORMS SELECTIVE ADDUCT WITH CYS125 OF RAT BETA-GLOBIN.

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PNU-142721E ((-)-6-chloro-2-[(1-furo[2, 3-c]pyridin-5-ylethyl)-thio]-4- pyrimidinamine) is a potent inhibitor of HIV-1 reverse transcriptase. Single dose excretion studies of [14C]PNU-142721 in the Sprague-Dawley rat revealed unusually long lived radioactivity associated with whole blood. Seven days post-dose, whole blood radioactivity was approximately 3 uM eq., more than 70-fold greater than corresponding plasma levels. In order to investigate the nature of the observed binding, rats were treated with increasing doses (10 to 100 mg/kg) or multiple doses (5 days) of [14C]PNU-142721 and blood samples were analyzed for radioactivity distribution. Results showed drug residues strongly associated with red blood cells; >90% of circulating radioactivity at 72 h post-dose was associated with red blood cells. Moreover, erythrocyte associated radioactivity increased additively with increasing dose or multiple doses. Fractionation and chromatographic analysis of protein from lysed erythrocytes showed distinct association of radioactivity with beta-globin. ESI-MS analysis of beta-globin isolated from a rat that had been treated with [14C]PNU-142721 showed the presence of a protein with a mass of 15976.1 Da, roughly 128 Da larger than the major form of rat beta-globin, suggesting addition of the chloropyrimidinamine functionality of PNU-142721. Amino acid sequence analysis of peptides generated from a tryptic digestion of the modified protein suggested that Cys125 of rat beta-globin was the site of modification. Further analysis of the radiolabeled peptides by MALDI-MS confirmed the sequence analysis and showed the chloropyrimidinamine adduct on cysteine. Comparison of rat and human hemoglobin sequences suggests that this reaction will not occur in humans because human beta-globin lacks a Cys residue at this position. However, it is unknown whether reactivity with protein thiols is a general feature of PNU-142721 metabolism.

1557 EQUINE CATECHOL ESTROGEN 4-HYDROXYEQUILENIN IS A SUBSTRATE AND AN INHIBITOR OF CATECHOL-O-METHYLTRANSFERASE.

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Redox and/or electrophilic metabolites formed from estrogen metabolism may play a role in estrogen carcinogenesis. 4-Hydroxyequilenin (4-OHEN) is the major phase I catechol metabolite of the equine estrogens equilenin and equilin, which are components of the most widely prescribed estrogen replacement formulation, Premarin®. Previously, we have found that 4-OHEN rapidly autoxidizes to an *o*-quinone *in vitro* and causes toxic effects including inactivation of human detoxification enzymes. 4-OHEN has also been shown to be a substrate for catechol-*O*-methyltransferase (COMT) in human breast cancer cells. In the present study, we demonstrated that 4-OHEN was not only a substrate of recombinant human soluble COMT *in vitro* with a K_m of 2.2 μM and k_{cat} of 4.7 min^{-1}, but it also inhibited its own methylation by COMT at higher concentrations. In addition, 4-OHEN was found to be an irreversible inhibitor of COMT-catalyzed methylation of the endogenous catechol estrogen 4-hydroxyestradiol (4-OHE2). 4-OHEN *in vitro* not only caused the formation of intra- and inter-molecular disulfide bonds as demonstrated by gel electrophoresis, but matrix assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS) also showed that 4-OHEN alkylated multiple residues of COMT. Peptide mapping experiments further indicated that Cys32 in recombinant human soluble COMT was the residue most likely modified by 4-OHEN *in vitro*. These data may suggest that inhibition of COMT methylation by 4-OHEN might reduce endogenous catechol estrogen clearance *in vivo* and further enhance toxicity.

1558 INHIBITION OF BAX TRANSLOCATION AND NECROSIS WITH BCL-XL OVEREXPRESSION IN A WELL CHARACTERIZED CELL CULTURE MODEL FOR TETRAFLUOROETHYLCYSTEINE-INDUCED NEPHROTOXICITY.

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Tetrafluoroethylcysteine (TFEC)-induced nephrotoxicity is initiated by the covalent modification of a relatively small number of defined mitochondrial proteins (1). The murine TAMH cell line has been characterized and faithfully reproduces TFEC-mediated renal injury and necrotic cytotoxicity with dose- and time-dependent inhibitions of both mitochondrial aconitase and α-ketoglutarate dehydrogenase target proteins (2). An early cytosolic to mitochondrial translocation of BAX (a proapoptotic BCL-2 family member) was also observed in TAMH cultures dosed with toxicologically relevant concentrations of TFEC (e.g. 250 μM). BAX translocations were confirmed immunocytochemically using confocal microscopy and with complementary immunoblot techniques. Effective and significant cytoprotection to supra-toxicological concentrations of TFEC (≤600 μM) were evident using TAMH stable transfectants overexpressing the BAX heterodimerization partner BCL-xL (c.f. vector control transfectants or parental cell line). Furthermore, BCL-xL overexpression limited the extent of BAX translocation to mitochondria in agreement with the cytoprotection observed in BCL-xL transfectants. In conclusion, effective cytoprotection to TFEC-induced necrosis is evident with BCL-xL overexpression and this appears to correlate well with the prevention of BAX subcellular relocation to mitochondria. Further data will also be presented which confirm our previous and related microarray studies (3) regarding the genomic responses of TAMH cells to TFEC-mediated intramitochondrial damage. 1. Cooper et. al., Biochem. Pharmacology 64, 553-564 (2002). 2. James et. al., Biochem. 41, 6789-6797 (2002). 3. Z-H Hu et. al., Toxicologist 66, LB66 (2002). Supported by NIH grants GM51916 (SAB), GM25418 (SDN), CA74131 (NF), American Cancer Society RPG-00-222-01-CDD (DMH) and NIEHS Center Grant P30ES07033.

1559 THE PROTECTIVE EFFECT OF FLAVONOIDS AGAINST OXIDATIVE DAMAGE INDUCED BY OCHRATOXIN A IN PROXIMAL TUBULAR CELLS.

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Flavonoids are present in many food commodities and have gained increasing interest in relation to disease prevention because of their antioxidant activity. Hence, oxidative stress leads to a variety of patho-physiological events. Worldwide, the mycotoxin ochratoxin A is a frequently found contaminant in human food and animal feeds and has been detected among others in cereals, beans and coffee. In humans, long-term exposure to ochratoxin A has been linked to a chronic kidney disease, denoted as Balkan Endemic Nephropathy (BEN). Induction of oxidative damage is one of the mechanisms involved in the renal toxicity of ochratoxin A. In the present study, GERP and LLC-PK1 cells were used as a model for proximal tubule cells, the target cells of ochratoxin A, to study the antioxidant properties of selected flavonoids. Following exposure to ochratoxin A (100 μM for 24 hours), the reactive oxygen species (ROS) production was measured using the fluorescent probe 2′, 7′-dichlorodihydrofluorescein diacetate (H2DCF-DA). The ROS scavenging ability of the tested compounds (in a concentration range of 0.1-100 μM) could be ranked in the following order: eriodictyol> quercetin > esculetin >> ebselen / naringin. The results showed that eriodictyol, quercetin and esculetin have antioxidant properties, efficient in the protection against the oxidative stress induced by the mycotoxin ochratoxin A. Therefore, the protective antioxidant properties of these compounds should be investigated further with respect to their ability to reduce ochratoxin A pathologies *in vivo*.