biotransformation enzymes. Although the mechanism(s) of induction by PB is not fully understood, current research is focusing on the role PB may play in altering the binding of nuclear proteins to critical DNA response elements. The objective of this study was to determine the effect of acute and chronic *in vivo* PB treatment on protein binding to various DNA regulatory elements including a TPA Response Element (TRE) and a Glucocorticoid Response Element (GRE). Rats received either a 70 mg/kg i.p. injection of PB for four days and sacrificed on day five, or 100 mg/kg PB i.p. 18 hours before sacrifice. Electrophoretic mobility shift assays (EMSAs) demonstrate no qualitative or quantitative differences between acute and chronic *in vivo* PB treatment regimens. Consistent with previous *in vitro* findings in other laboratories PB treatment *in vivo* enhanced AP-1 binding activity to a TRE. In contrast, PB had no effect on protein binding to the GRE. Our results show that AP-1 binding occurs following acute PB exposure *in vivo* and persists during chronic treatment, thus providing further evidence to support a role of AP-1 in the induction mechanism of PB. *Acknowledgements:* A.L.R. supported by NIEHS Training Grant 5 T32ES07266. Research supported in part by Sigma Xi Grant-in-Aid of Research Award (ALR).

306 CHARACTERIZATION OF CYTOCHROME P450 AND GLUTATHIONE TRANSFERASE ACTIVITIES IN SV40-IMMORTALIZED UROEPITHELIAL CELL LINES

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An *in vitro/in vivo* transformation system has been developed as a model for bladder tumorigenesis (Bookland et al, Cancer Res 52: 1606–1614). SV40-immortalized human uroepithelial cells are exposed to putative carcinogens and then implanted into athymic nude mice to test for tumorigenesis. Studies with 4-aminobiphenyl (4-ABP) demonstrated that one cell line was sensitive to chemical-induced transformation, SV-HUC-PC, and another line, SV-HUC-BC, was refractory. We are currently testing this system as a model to identify occupational carcinogens and develop biomarkers of effects. This study examined the P450-dependent metabolism, glutathione transferase, and the effects of chemicals on DNA synthesis and repair in SV-HUC-PC and SV-HUC-BC. Activities for CYP1A1/1A2, CYP3A and CYP2B1/2B2 were estimated by determining o-deakylation of ethoxy-, benzoxy- and pentoxy-resorufin, respectively. Coumarin hydroxylase and p-nitrophenol hydroxylase were used to estimate CYP2A and CYP2E1, respectively. SV-HUC-PC microsomes had 5 fold greater CYP1A1/1A2 activity and 2 fold higher CYP3A activity than SV-HUC-BC. CYP2B1/2B2- and CYP2A-activities and glutathione transferases were not different between the two cell lines. DNA synthesis and repair, by BrdU incorporation, was not different between the two lines when MMNG or other reactive metabolites were tested; however, SV-HUC-PC was more sensitive to n-nitrosodimethylamine, 4-ABP and MOCA. The data demonstrate that while these cells have retained form-specific P450 activities, SV-HUC-PC has greater CYP1A1/1A2 and CYP3A activities.

307 EVALUATION OF THE ROLE OF CYP2C19 GENOTYPE IN BLADDER CANCER

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CYP2C19 has been shown to be polymorphic in humans. The enzyme coded by this gene is responsible for the metabolism of the anticonvulsant drug *S*-mephenytoin and a variety of other drugs. However, its role in the metabolism of carcinogens and in cancer risk has not been investigated. We have reported two major genetic defects in *CYP2C19* (*m1* and *m2*) that are responsible for the polymorphism and have developed PCR-based genotypic assays. The *m1* defect is a G→A mutation that creates an aberrant splice site in exon 5, and the *m2* mutation is a G→A mutation that creates at stop codon in exon 4. To investigate the role of *CYP2C19* genotype in bladder cancer, we used the PCR-RFLP assays to analyse 221 bladder cancer cases and 200 sex, race, and age frequency-matched controls for the *m1* and *m2* defects. In Caucasian controls, the frequency of the *m1* allele was 0.15. The *m1/m1* homozygotes comprised 1% of Caucasian controls, but 4% of Caucasian cases. In previous studies, the *m2* allele comprised 15–30% of the defective alleles among Orientals. However, *m2* was not observed in this study of a total of 392 Caucasian cases and controls nor in 29 African-American cases and controls. Among Caucasians, the risk of bladder cancer was 3.9-fold (95% CI = 0.8–18.5, p = 0.07) higher among persons with the *m1/m1* (poor metabolizer) genotype relative to persons with the *wt/m1* or *wt/wt* (extensive metabolizer) genotypes. Stratifying by smoking status reveals no increased risk of the *m1/m1* genotype among non-smokers (OR = 0.99, 95% CI = 0.3–30, p = 0.99) and an increased risk among smokers (OR = 6.0, 95% CI = 0.7–48, p = 0.06) although there was no evidence of a dose-response among different levels of smokers. These preliminary results suggest a possible association between *CYP2C19* genotype, environmental exposures, and bladder cancer risk.

308 Ah-RECEPTOR DEPENDENT MODULATION OF GENE EXPRESSION BY AGED AND DILUTED SIDESTREAM CIGARETTE SMOKE (ADSS)

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Sidestream smoke is known to induce CYP 1A1 gene expression in rat and mouse lung as confirmed by increased enzyme activity and increased protein and message levels. The exact mechanism of how such induction occurs is based in logical speculation rather than experimentally supported proof. In this study we examined the mechanism of gene expression induction by ADSS in mouse hepatoma hepa1c1c7 (hepa1) cells. Recombinant hepa1 cells stably transfected with a luciferase gene under control of several dioxin responsive elements (DREs) were exposed to ADSS and the luciferase gene expression was examined. Induction of gene expression was observed with whole ADSS. No induction was observed with filtered ADSS suggesting that the particulates in the smoke are the inducing agent(s). α-naphtoflavone, an Ah-receptor antagonist, suppressed ADSS mediated induction of the luciferase gene. Nuclear protein extract from cells exposed to ADSS was found to bind to DRE as detected by gel shift assay. These findings strongly support the hypothesis that the Ah-receptor mediates induction of gene expression by ADSS. Supported UCTRDRP grant #4RT-0213.

309 PURIFICATION OF RAT LIVER NUCLEAR FACTOR THAT BINDS TO 5′-UP STREAM REGION OF CYP 1A1 GENE

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A negative regulatory element (NRE) has been localized in the 5′-up stream region of the CYP1A1 at −843 to −746 bp from the site of transcription. This element has >80% sequence identity to the 26 bp fos/jun-octamer binding motif of the c-myc NRE. Here we describe the purification of Oct-1 factor from rat liver nuclei and its effects on CYP1A1 expression. Specific binding to an octamer containing motif in the 5′-up stream region of CYP1A1 was used in the purification of the factor to near homogeneity. The purification procedure included conventional chromatographic methods and DNA site recognition affinity chromatography. We have also demonstrated the suppression of reporter gene activity in hepatoma cells in to which a fusion construct containing the octamer binding sequence had been transfected. From the data we conclude that we have purified the nuclear factor Oct-1 that has a role in the down regulation of the rat CYP1A1 gene.

310 THE POTENTIAL INFLUENCE OF INTERINDIVIDUAL VARIATION IN PHASE I AND PHASE II METABOLISM OF BENZENE ON BENZENE TOXICITY

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In humans, myelotoxicity of benzene (BZ) likely results from oxidative metabolism to reactive products. However, susceptibility to toxic effects of BZ may be related to a balance between activation (Phase I) and detoxication (Phase II) reactions. In the present study, we have estimated kinetic parameters of BZ oxidation and two detoxication reactions, phenol (PH) sulfation and hydroquinone (HQ) glucuronidation, in liver samples from ten humans. In these samples, initial rates of oxidative metabolism of benzene catalyzed by cytochrome P450 (CYP) 2E1 varied from 0.402 to 5.252 nmoles/mg/min. Initial rates of PH sulfation varied 3-fold (range: 0.309 to 0.919 nmole/mg/min), as did initial rates of HQ glucuronidation (range: 0.101 to 0.281 nmole/mg/min). *In vitro* bone marrow studies have demonstrated that HQ can be oxidized to 1,4-benzoquinone, a highly lipophilic compound that undergoes extensive protein and DNA binding. That reaction is enhanced in the presence of PH. Therefore, a physiologically-based pharmacokinetic model that incorporates measured rates of oxidation and conjugation reactions was developed in order to predict steady-state concentrations of PH and HQ in blood of the ten human donors during a low BZ exposure. Predicted steady-state concentrations of PH and HQ varied 8-fold and 3.4-fold, respectively. In all simulations, predicted concentrations of HQ exceeded those for PH, and in all but one human the difference was greater than 10-fold. Predicted concentrations of HQ at

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