

1811 CHANGES IN ESTROGEN METABOLISM IN CATECHOL-O-METHYLTRANSFERASE (COMT) DEFICIENT MICE ARE ASSOCIATED WITH INCREASED DEVELOPMENT AND ALTERED GENE EXPRESSION IN THE MOUSE MAMMARY GLAND.

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Estradiol (E2) has two catechol estrogen (CE) metabolites that are inactivated by COMT, a phase II biotransformation enzyme. Studies in our lab demonstrated that a low activity polymorphism for COMT is associated with increased risk for breast cancer and that COMT inhibition leads to increased oxidative DNA damage in MCF-7 cells. Additional studies show that mammary gland cells from COMT knockout (KO) mice metabolize E2 to CEs, but do not produce methoxy estrogen metabolites, which are thought to be anti-mitogenic. Additionally, preliminary experiments show that serum E2 levels (pg/mL) are higher ($p \leq 0.04$) in the KO mice (42.1 ± 13.4 ; $n=5$) than in their wild-type (WT) counterparts (7.0 ± 1.9 ; $n=3$). CEs bind to the estrogen receptor, are estrogenic, and are precursors of estrogen quinones that cause DNA damage. Thus, we hypothesized that mammary gland development in the KO mice would be affected by the observed alterations estrogen metabolism. To test this hypothesis, mammary glands were excised from 4 week old KO and WT mice and subjected to whole mount examination of the epithelial tree. Ductal branching was quantified by counting terminal end buds and RNA was isolated for microarray analysis of gene expression. Our results show that the KOs have ($p \leq 0.02$) more terminal end buds per gland (34.30 ± 5.030 ; $n=3$) than WTs (19.8 ± 6.9 ; $n=5$), suggesting greater development. Microarray analysis shows that 15 genes were significantly up-regulated in the KOs (fold change range: 1.5-6.8; $p \leq 0.05$). The up-regulated genes have been associated with cell signaling, growth/proliferation and cancer, suggesting that the alterations in estrogen metabolism might result in changes in gene expression in the mammary gland and may contribute to altered development. (Supported by NIH grants R01 CA77550, T32 ES07141, and P30 ES03819).

1812 INVESTIGATION OF OCTAMETHYLCYCLOTETRAILOXANE (D4) AND DECAMETHYLCYCLOPENTASILOXANE (D5) AS DOPAMINE D2-RECEPTOR AGONISTS.

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Oncogenicity studies of D4 and D5 have shown uterine adenomas and adenocarcinomas, respectively. Binding of dopamine receptor agonists to dopamine D2-receptors on pituitary lactotrophs inhibits the process of prolactin secretion. In the rat, decreased circulating levels of prolactin induces a decrease in circulating progesterone and consequently, an elevated estrogen:progesterone ratio. The neoplastic effects of dopamine receptor agonists on the uterus have been attributed to this apparent estrogen dominance. Experiments were conducted to evaluate the potential for D4 and D5 to modulate pituitary prolactin secretion as dopamine D2-receptor agonists. Utilizing an *in vitro* cell line, derived from a rat pituitary tumor (MMQ, ATCC #: CRL-10609), 10 μ M D4 and D5 were each shown to decrease maitoxin-induced prolactin release by 55% without affecting viability. An *in vivo* model was then used to assess serum prolactin levels in reserpine-treated female Fischer 344 rats following 6-h vapor inhalation exposure to 700ppm D4 or 160ppm D5. In this model, serum prolactin levels were decreased 88% by 700ppm D4 and 50% by 160ppm D5 relative to reserpine control. Pretreatment with sulpiride, a dopamine receptor antagonist, blocked the effect of D4 and D5 suggesting that these cyclic siloxanes were acting on the pituitary as dopamine D2-receptor agonists *in vivo*. These results and the known species differences in reproductive physiology provide support for a potential mode of action that is not relevant humans. (This work was sponsored by the Silicones Environmental, Health and Safety Council of North America)

1813 TRANSPLACENTAL AND POSTNATAL EXPOSURE OF AIDS DRUGS ZIDOVUDINE (AZT) AND LAMIVUDINE (3TC) IN C3B6F₁ TRP53(+/-) TRANSGENIC MICE.

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AZT/3TC antiviral drug combinations are given during pregnancy to reduce maternal-fetal HIV transmission. AZT is genotoxic in fetal mice and monkeys and carcinogenic in mice. We assessed a new C3B6F₁trp53(+/-) p53 haplodeficient transgenic mouse model to be used for cancer bioassays. These mice, produced by

mating Taconic C57BL6(N12)trp53(-/-) males and C3H females, possibly have similar tumor profiles to B6C3F₁ mice. Haplodeficient C3B6F₁trp53(+/-) and wild-type C3B6F₁trp53(+/-) mice were dosed with 0, 40, 80, 160 mg/kg AZT or 160 mg/kg AZT combined with 100 mg/kg 3TC, by gavage in aqueous methylcellulose/polysorbate 80 (2/0.1%), transplacentally from GD12 to GD18 then postnatally from PND 1 to PND 28. P53 deficient mice are susceptible to fetal and neonatal mortality particularly when exposed to genotoxic compounds *in utero*. In a previous study using perinatal C57BL6(N5)trp53(+/-) mice dosed with 200 mg/kg AZT, we obtained relatively low pup survival in both control and treated groups (75 & 17% respectively at PND-28). In contrast, C3B6F₁trp53(+/-) were more robust. Survival in these pups was >95% and >85% for the control and dosed groups respectively and was greater for haplodeficient than for wild type mice. The AZT and AZT/3TC treatment produced only small (<10%) reductions in body weight gain, but mice from the AZT/3TC dose groups showed increased *hprt* mutation frequency when evaluated at PND 28. In adult humans hepatic AZT glucuronidation by UDP-glucuronosyltransferase (UGT) is a major detoxification pathway. In contrast, liver from C3B6F₁trp53(+/-) and C3H mice expressed very low levels UGT activity towards AZT (0.2 - 1.5 pmol/min/mg microsomal protein; human and rhesus monkey liver activities ranged between 1.5 - 2.5 nmol/min/mg microsomal protein), and only low levels of AZT-glucuronide were detected in serum from AZT treated mice. Since human neonates also express low levels of hepatic UGT activity, the C3B6F₁trp53(+/-) mouse may be a good model for evaluating risk of perinatal AZT exposure.

1814 EFFECT OF A COMPLEX MIXTURE FROM COAL TAR - SRM 1597 ON THE METABOLIC ACTIVATION OF CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN CHINESE HAMSTER V79 CELLS.

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A complex mixture of polycyclic aromatic hydrocarbons (PAH) extracted from coal tar, Standard Reference Material (SRM) 1597, has been shown to initiate tumor formation in mouse initiation-promotion assays in our laboratory (*Carcinogenesis*, 2001, 22:(7) 1077-1086). To understand the role of SRM 1597 on PAH activation and DNA adduct formation, we examined the effects of SRM 1597 on the metabolic activation to DNA-binding derivatives of two carcinogenic PAH, benzo[*a*]pyrene (BP) and dibenzo[*a,h*]pyrene (DBP). Chinese hamster V79 cells expressing either human cytochrome P450 (CYP) 1A1 or CYP1B1 were used to determine the PAH-DNA adduct levels on exposure to SRM 1597, BP, DBP or co-treatments of SRM 1597 and BP or DBP. SRM 1597 inhibited BP-DNA adduct formation through the entire exposure time in the human CYP1A1 expressing cells. On the contrary, V79 cells expressing human CYP1B1 were unable to metabolize SRM 1597 or co-treatments of SRM 1597 and BP or DBP to DNA-binding metabolites. However, both CYP1A1 and CYP1B1 expressing cells metabolized BP and DBP to their respective DNA-binding metabolites. In order to assess the ability of SRM 1597 to inhibit the activity of CYP enzymes, ethoxyresorufin *O*-deethylase assay was performed using microsomes isolated from V79 cells expressing CYP1A1 and CYP1B1. Competitive inhibition of resorufin product formation on exposure to SRM 1597 was observed in microsomes from V79 cells expressing both human CYP1A1 and CYP1B1. The results from this study suggests that the relative risk that PAH mixtures pose to humans may be dependent on the ability of the complex mixture to competitively inhibit the metabolic activation of carcinogenic PAH by the induced CYP enzymes and decrease DNA binding metabolites. Supported by NIH grant CA28825.

1815 COMPETING ROLES OF ALDO-KETO REDUCTASE 1A1 AND CYP1A1/CYP1B1 IN THE METABOLIC ACTIVATION OF (+/-)-BENZO(A)PYRENE-7, 8-DIOL IN HUMAN BRONCHOALVEOLAR CELLS: INFLUENCE OF CYP INDUCTION.

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Human aldo-keto reductases (AKR1A1, AKR1C1-AKR1C4) catalyze a novel pathway of (+/-)-Benzo[*a*]pyrene-7, 8-diol (BP-7, 8-diol) activation which leads to BP-7, 8-dione that differs from the cytochrome P450 (CYP1A1/CYP1B1) pathway which leads to anti-benzo[*a*]pyrene-diol epoxide (anti-BPDE). The roles of AKR1A1 vs. CYP1A1/CYP1B1 in the activation of BP-7, 8-diol were equivalent in bronchoalveolar cell extracts containing 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (TCDD)-induced CYP1A1/CYP1B1 and stably expressed AKR1A1, and were dependent on the redox-state (NAD⁺ : NADPH). This study compares the roles of