express ΔAhRmt, XRE-driven luciferase activity was still inducible by TCDD. Taken together these data would indicate that, depending on which genes were investigated, overexpressed ΔAhRmt varies in its ability to repress the transactivation function of the AhR pathway. In HeLa cells transiently expressing AhR527, the co-transfected basal XRE-driven luciferase activity was decreased ~1.6 fold. In addition, when HeLa cells were treated with geldanamycin, an antibiotic which decreases the cellular level of AhR, the basal XRE-driven luciferase activity in pGud/Luc 6.1-stably-transfected HeLa cells decreased in a similar manner. These results indicate that the AhR pathway is constitutively active in HeLa cells.

334 INTERACTION BETWEEN THE Ah RECEPTOR (AhR) AND NF-κB SIGNAL TRANSDUCTION PATHWAY.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a potent immune suppressant, endocrine disruptor, tumor promoter and teratogen. These responses are mediated by the AhR which is a ligand-activated SHLH protein. The wide range of physiological responses caused by TCDD strongly suggests that the AhR interacts with many cellular regulatory pathways. In this study, we show the AhR physically associates with the p65 component of the NF-κB complex. This interaction was observed in TCDD treated COS7 cells co-transfected with AhR and p65. This association is present both in cytosolic fraction detected by co-immunoprecipitation, and in the nucleus, as a complex binding to the κB enhancer sequence. TCDD down-regulates the NF-κB gene expression by reducing the binding of NF-κB complex to the κB site and functionally down-regulates the IL-6 CAT reporter gene expression mediated by NF-κB. Interestingly, NF-κB was found to reciprocally down-regulate the AhR function. Increasing gene expression of p65 decreased the AhR-mediated CAT reporter gene expression. Since NF-κB is known to be involved in a broad range of gene expression, the interactions between the NF-κB and AhR signal transduction pathways may be important in the plethora of toxic effects of TCDD. Supported in part by NIEHS Center Grant #ES05002.

335 HEPATOCARCINOGENESIS IN A SPRAGUE-DAWLEY RAT INITIATION/PROMOTION MODEL FOLLOWING DISCONTINUOUS TREATMENT WITH TCDD.
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In rodent models, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a multi-site, trans-species carcinogen in both sexes. One of the characteristics of tumor promotion is its reversibility upon withdrawal of the promoting agent. The aim of the current study was to investigate the effect of discontinuous treatment regimens on the promotion of hepatocarcinogenesis by TCDD. Female Sprague-Dawley rats were either initiated with 175mg diethylnitrosamine/kg body weight at 10 weeks of age or received saline alone. Two or 18 weeks after initiation, animals began treatment with TCDD at a daily averaged dose of 125 ng/kg/day for up to 60 weeks. Control animals received corn oil alone. For some groups, after 30 weeks of TCDD exposure, TCDD treatment was stopped and the animals subsequently received corn oil for the remainder of the study. Continuous treatment with TCDD for 60 weeks induced altered hepatic foci formation, cell proliferation, and tumor incidence. Cessation of treatment with TCDD led to a reduction in altered hepatic foci development, reversal of TCDD induced changes in cell proliferation, and lower incidence of hepatic tumors. The modulation of hepatocarcinogenesis by discontinuous treatment with TCDD support the hypothesis that changes in the promotional environment in the liver may alter the ability of subsets of foci to progress to tumors.

336 O3-INDUCED ALDEHYDE PRODUCTION: THE INFLUENCE OF LUNG SURFACE LINING LAYER SUBSTRATE CONDITIONS.
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Inhaled O3 likely does not produce acute toxicity via direct interactions with the lung epithelium. Chemical reactions with surface lining layer (SLL) constituents influence the O3 flux rate, limit solute O3 diffusion, and produce SLL-derived products that initiate a cascade leading to cellular pathophysiology. Although O3 quickly reacts with many non-lipid SLL constituents, lipid oxidation products (LOP) are produced during exposure. To delineate specific aspects of LOP formation, we measured the yields of hexanal (C6, from unsaturated fatty acid oxidation) and the ozonation specific aldehydes heptanal (C7) and nonanal (C9) as a function of ascorbic acid (AH3) concentration during O3 exposure of isolated rat lungs, rat bronchoalveolar lavage fluid (BALF), and egg phosphatidylcholine liposomes (EggPC). Exposures were conducted under quasi-steady-state conditions and O3 uptake (U) quantified. Results: 1) O3 reactive absorption by AH3, uric acid, and albumin exceeded EggPC and GSH. 2) Addition of lung and BALF exposures in a time and O3-dependent manner but represented only a minor proportion of U. 3) Reducing SLL [AH3] lowered U but increased C6, C7, & C9 yields. 4) Addition of AH3 to EggPC increased U but reduced aldehyde yields. 5) Addition of 10% linoleic acid to EggPC increased U and C6. Conclusions: Within the SLL milieu, direct O3 reaction with UFA occurs and produces LOP in small yield, related to AH3 availability. Variations in SLL conditions may produce complex relationships between aldehyde production and O3 dose. Thus, estimating O3 dose based on product formation should be viewed with appropriate caution. (NIH HL5469602 & HEI 91-7)

337 OXIDATION OF LUNG PROTEINS IN RATS EXPOSED TO INHALED NITRIC OXIDE AND HYPEROXIA.
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Inhaled nitric oxide (NO), commonly administered with 100% oxygen, is emerging as a therapy for treatment of pulmonary hypertension associated with lung diseases. The present study examines exposure to inhaled NO/ hyperoxia and oxidation of specific proteins, which could be used as biomarkers for monitoring potentially adverse effects of inhaled NO therapy. Male Fischer 344 rats were placed in 100% NO at FiO2>0.95 for 48 hr, with controls exposed to FiO2=0.95 or room air. Although no rats died prematurely, the rats exposed to hyperoxia or inhaled NO/hyperoxia had marked respiratory distress. The animals exposed to NO and hyperoxia showed markedly greater protein concentrations in their lavage fluids than did either the animals exposed to hyperoxia alone or the air-breathing controls. To assess protein oxidation, proteins from bronchoalveolar lavage (BAL) and lung homogenates were derivatized with 2,4-dinitrophenylhydrazine (DNPH), separated by gel electrophoresis, transferred, and DNPH-reactive protein carbonyls detected with anti-DNPH antibodies. N-terminal amino acid sequencing of a unique DNPH-reactive protein at MW 54kD, found only in the BAL from NO/hyperoxic rats, matched Vitamin D-binding protein precursor. This protein is responsible for transport of Vitamin D and demethylation of actin at sites of tissue necrosis, suggesting a possible role in resolution of lung injury. Supported by Abbott Laboratories.

338 MICROFILAMENTS AS A MARKER FOR MACROPHAGE FUNCTION IN ANIMALS EXPOSED TO OXIDANT GASES.

Microfilaments have been shown to be important mediators in macrophage spreading, phagocytosis, and formation of pseudopod extensions. Sprague Dawley rats were exposed to filtered air (FA), 0.8 ppm ozone (O3) or a combination of 14.4 ppm NOx/0.8 ppm O3 to determine whether macrophage function is altered via microfilament reorganization. Animals were exposed for 6 hr/day for 1, 2, 7, 5 and 8 wks. Macrophages (MØs) recovered from bronchoalveolar lavage fluid were analyzed for their chemotactic and phagocytic activity. Control lavage supernatant previously incubated with zymosan particles served as a MØ chemotactant for the chemotaxis assays. MØs incubated with serum-coated 1.0µm fluorescent microspheres were used to estimate the phagocytic ability. Microfilament staining for G- and F-actin was visualized with Oregon-green labeled phalloidin and rhodamine labeled DNsase I, respectively. Results showed that total cell number and viability in the O3-exposed group were comparable to FA during exposure. Neutrophil numbers significantly increased in the lavage fluid with 1 and 2d of O3 and 8wks of O3/NOx exposure. No change in eosinophil, basophil or epithelial cell populations were observed in any of the exposure regimens. The migratory and phagocytic ability (FA-uptake) in the O3-exposed group did not change compared to FA, whereas with O3/NOx a dramatic increase in the ability to phagocytize particles and a decrease in chemotactic ability was found. Microfilaments in animals exposed to O3 showed the same staining distribution for G-actin as FA, but a more abundant staining for F-actin. The O3/