208 OXIDATIVE STRESS CAUSED BY GLUTATHIONE SYNTHESIS INHIBITOR BUTHIONINE SULFOXIMINE RESULTS IN GENOME REARRANGEMENTS IN MICE.

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Oxidative stress has been associated with cancer and other diseases, such as neurodegenerative, bronchopulmonary and cardiovascular diseases, to name a few. A number of xenobiotics, including cigarette smoke, diesel exhaust, and certain pharmaceutics, cause oxidative stress. L-Buthionine-S, R-sulfoximine (BSO) is used to pharmacologically induce oxidative stress in research studies on the effects of oxidative injury. BSO induces endogenous oxidative stress by irreversibly inhibiting g-glutamylcysteine synthetase, an essential enzyme for the synthesis of glutathione (GSH), a major cellular thiol antioxidant. We tested whether lowering cellular antioxidant capacity by BSO can lead to genomic rearrangements that may play a role in carcinogenesis or other genetic disease. We determined the frequency of DNA deletions, the levels of oxidative DNA damage measured by 8-OH deoxyguanosine (8-OHdG), and GSH levels, in mouse embryos exposed to BSO via drinking water given to pregnant dams. A low (2 mM) BSO dose treatment resulted in elevated frequencies of DNA deletions, higher 8-OHdG levels, but no changes in GSH concentration. A 10-fold higher (20 mM) BSO dose caused a 1.6-fold increase in DNA deletion frequency, 5-fold higher levels of 8-OHdG and depleted GSH to 30% of control level. The thiol antioxidant N-acetyl-L-cysteine had a protective effect against BSO treatment-induced DNA deletions. This study showed that endogenous oxidative stress results in genomic rearrangements that may play a role in development of cancer.

209 INHIBITION OF HSP90α CHAPERONE ACTIVITY BY 4-HYDROXY-2-NONENAL.

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Intracellular concentrations of 4-hydroxynonenal (4-HNE), malondialdehyde (MDA) and other lipid-derived aldehydes are elevated under conditions of oxidative stress. These aldehydes have a documented ability to modify proteins, thereby disrupting function. Proteomic analysis of rat liver subcellular fractions has demonstrated consistent modification of heat shock protein 90 (Hsp90) by 4-HNE and MDA in a model of chronic alcoholic liver disease. A series of *in vitro* studies were conducted to test the hypothesis that these lipid aldehydes interfere with Hsp90 chaperone activity. Purified recombinant Hsp90α was treated with increasing concentrations of 4-HNE and MDA, resulting in a corresponding increase in the severity of protein modification by these aldehydes, demonstrated by immunoblot. 4-HNE protein adducts were confirmed and located by tryptic digest and HPLC-MS/MS peptide analysis. Pretreatment of Hsp90α with 10 μM 4-HNE resulted in a 68% inhibition in chaperone activity using a firefly luciferase refolding system, while the chaperone was completely resistant to inactivation following pretreatment with 10 μM MDA. Pretreatment with 10 μM 4-oxononenal (4-ONE), which is highly reactive towards Cys residues, also resulted in significant inhibition of Hsp90-assisted protein refolding. Because MDA is comparatively less reactive toward Cys than 4-HNE or 4-ONE, these results suggest a Cys specific mechanism. Finally, disappearance of luciferase was measured throughout the duration of the timecourse; however, rapid substrate degradation does not account for the observed 4-HNE-mediated inhibition of protein refolding, as demonstrated experimentally. The data presented here demonstrate modification of Hsp90 by 4-HNE and MDA in the livers of rats fed a chronic high fat/ethanol diet, and inhibition of Hsp90 chaperone-like activity by Cys-reactive lipid aldehydes. This work was supported by NIH/NIAAA RO1AA09300 and NIH/NIEHS RO1ES09410 (DRP), NIH/NIEHS F32 ES11937 (JAD), and NIH/NIAAA F31 AA014308 (DLC).

210 HIGH FAT DIET DIFFERENTIALLY AND SITE-SPECIFICALLY ALTERS LUNG GLUTATHIONE AND ACUTE CYTOTOXICITY IN MALE AND FEMALE MICE.

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The average American consumes 37% of their calories from fat. Very little is known about the effect of high fat diet on lung cells. To evaluate the effect of diet on the lung, male and female C57Bl6/J mice were fed a high fat diet (58% kcal fat) or a matching low fat diet (10.5% kcal fat) beginning at 4 wks of age. Body weight and intra-abdominal fat pad weight (8-12 wks of age) were significantly increased by the high fat diet($P < 0.01$). Intrapulmonary and extrapulmonary airways were microdissected for analysis of glutathione (GSH; measured using HPLC) and total glutathione S-transferase activity (GST; turnover of CDNB substrate). GST activity did not vary significantly between high and low fat groups, by airway level or by sex. However, GSH levels were significantly elevated by high fat diet in intrapulmonary airways of female mice ($P<0.01$). GSH levels in extrapulmonary airways of females and both airways in the male were unaffected by diet. Lungs from mice fed a high fat diet were more fibrous on dissection. Collagen deposition defined by picrosirius red staining was increased in mice fed a high fat diet. Additional animals were challenged with naphthalene (NA) i.p. to assess pulmonary responses to injury at 1 and 24 hrs. Female animals were more susceptible than males to NA injury. High fat diet reduced the amount of NA injury in the large intrapulmonary airways of female mice but had no effect in males. Male mice increased intrapulmonary GSH levels in response to NA injury and this was unaffected by diet. However, female mice were incapable of increasing their 1 hr GSH levels in response to NA injury in either the high or low fat diet groups. We conclude that a high fat diet changes the steady-state biology of the lung and that changes in response to diet and/or injury challenge are airway level and sex-specific. Support: California Tobacco-Related Diseases Program grant 12IT-0191, NIH ES013066, ES04311, ES05707

211 MOLECULAR PATHOLOGICAL ANALYSIS OF HEPATOCARCINOGENESIS IN MICE TREATED WITH DICYCLANIL.

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In order to clarify the mechanism of hepatocarcinogenesis induced by dicyclanil (DC), molecular pathological analyses in the liver of mice were performed. At Experiment I, a large-scale cDNA microarray and the validation of RT-PCR were performed using the liver of mice given the diet containing 0 and 1500 ppm of DC for 2 weeks. The gene expressions of metabolism- or oxidative stress- related genes, such as Cyp1a, Aldh1a1, and Txnrd1, were predominantly up-regulated in the liver of mice treated with DC. At Experiment II, in addition to histopathological analyses, stress pathway-specific small-scale microarray and the validation of real-time RT-PCR were performed in the liver of mice using a two-stage hepatocarcinogenesis model, in which they were given 1500 ppm of DC for 7 weeks after injection of dimethylnitrosamine (DMN) with a partial hepatectomy (PH). The number of gamma-glutamyltransferase (GGT)-positive cell and PCNA Labeling Index were significantly increased in the liver of the DMN+DC+PH group that showed fluctuations of gene expressions of Cyp1a, Txnrd1, Ogg1, and Gadd45a related to oxidative stress or DNA damage/repair. To evaluate oxidative stress and DNA damage induced by the treatment of DC, 8-hydrixy-2-deoxyguanosine (8-OHdG) was measured in the liver DNA of two-stage hepatocarcinogenesis model mice given DC for 13 weeks (Experiment III). In addition to the formation of GGT positive foci, significant increases of 8-OHdG were observed in the DMN+DC+PH groups. These results suggest that DNA damage due to oxidative stress is partially associated with the mechanism of DC-induced hepatocarcinogenesis on mice.

212 ROLE OF NITRIC OXIDE IN DIESEL EXHAUST PARTICLE-INDUCED GENOTOXIC AND MUTAGENIC ACTIVITIES IN THE RAT LUNG.

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Exposure of rats to diesel exhaust particle (DEP) has been shown to induce the formation of inducible nitric oxide synthase (iNOS) and elevate nitric oxide (NO) production by alveolar macrophages (AM). DEP are known to induce pulmonary inflammation and increase pulmonary susceptibility to infection through altered metabolic function and secretion of pro- and anti-inflammatory cytokines. The present study examines the role of NO in DEP-altered P450 activities which mediate genotoxic and mutagenic effects in the lung. Male Sprague-Dawley rats were intratracheally (IT) instilled with saline, DEP (35 mg/kg) or the DEP organic extract (DEPE). To illustrate the role of iNOS, another group of rats was treated with an iNOS inhibitor, aminoguanidine (AG, 100 mg/kg), by i.p. injection 30 min prior to and 3, 6 and 9 h after IT exposure. At 1 day post exposure, DEP-induced genotoxicity and lung-S9 dependent mutagenicity were monitored via comet assay and the Ames test with *S. typhimurium* YG1024, respectively. The results show that AG treatment markedly inhibited DEP-induced NO production by AM without affecting iNOS levels. DEP-exposed AM exhibited significant DNA damage compared to controls. Both DEP and DEPE induced CYP1A1 activity, which was significantly reduced by AG. However, DEP and DEPE attenuated CYP2B1 activity that was not altered by AG. CYP1A1 and CYP2B1 supersomes activated 2-aminoanthracene (2-AA) mutagenicity in the Ames test, suggesting that both CYP isoforms were involved in 2-AA activation. DEP- and DEPE-S9, although containing less CYP2B1, activated 2-AA to a similar extent as the control. AG treatment significantly lowered DEP- and DEPE-S9, but not control-S9, dependent activa-

tion of 2-AA mutagenicity. These results demonstrate that DEP exposure induces genotoxicity and mutagenicity in the lung. The organic component of the particle is responsible for DEP-induced CYP1A1, while NO plays a major role in DEP-induced mutagenic effects in the lung by regulating CYP1A1 activity.

213 CONTRIBUTION OF REACTIVE OXYGEN SPECIES TO PARA-AMINOPHENOL-INDUCED CYTOTOXICITY.

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Para-aminophenol (PAP), a metabolite of N-acetyl-para-aminophenol (APAP), causes toxicity in the renal proximal tubule. LLC-PK1 cells show a concentration-dependent loss of viability following a 4-hour PAP treatment with an EC50 of 0.1mM. Oxidation of PAP is a key step in its cytotoxicity, however the mechanism is yet to be determined. Previously we have shown that pre-incubation and/or co-incubation with ascorbic acid or glutathione (GSH) protects cells from PAP toxicity. This suggests that PAP may be causing the formation of reactive oxygen species (ROS). The present studies were designed to determine the presence of ROS and to identify the species that may be produced. LLC-PK1 cells were pre-treated with varying concentrations of catalase (0-100 units/ml), hydroxyl radical scavengers [DMTU (0-50mM) and mannitol (0-75mM)], or metal chelators [1, 10-phenanthroline (0-0.25mM), and bathocuproine (0-10 mM)] for 30 minutes. We then added 0, 0.1mM PAP or 0.15mM PAP, incubated for an additional 4 hours and measured cell viability 20 hours later. Menadione was used as a positive control as it undergoes redox cycling leading to formation of superoxide anions. Toxicity due to PAP was attenuated by all pre-treatments. These data suggest that PAP cytotoxicity is at least partially due to generation of reactive oxygen intermediates. Supported by NIH R15GM065196.

214 ENVIRONMENTAL BASIS OF NEURODEGENERATION (ND) AND AGING: DIBROMOACETONITRILE (DBAN) INDUCES PROTEIN OXIDATION, INHIBITS PROTEASOMAL ACTIVITY AND ALTERS CYTOSOLIC PROTEOME IN NEUROGLIAL CELLS.

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Our goal is to investigate the role of environment in fetal basis of ND/aging. Epidemiological studies indicated that *in utero* exposure to drinking water disinfectant byproducts (DBP) such as DBAN causes susceptibility to adverse pregnancy outcomes including CNS anomalies. Our previous studies indicated that DBP cross the placenta, retain in fetal brain cortex and hippocampus, where it induces oxidative stress (OS) and apoptosis. OS-mediated oxidation of amino acid side chains (protein carbonyls) are increased in ND/aging. Inefficient degradation of carbonylated protiens by proteasomes result in the accumulation of proteolysis resistant protein aggregates in ND/aging. We hypothesize that DBP-induced OS causes protein carbonylation, accumulation of oxidized proteins and proteasomal inhibition leading to neuronal loss. Neuroglial cells (Rat C-6 glioma) were exposed to DBAN (0-400 ppb) for 24 or 48 h. The results indicated that DBAN induced a concentration and time-dependent increase (128-136% of control, $p < 0.05$) in carbonylated proteins and a decrease (65-58% of control) in proteasomal activities. DBAN-induced changes in cytosolic proteome was evaluated by 2D gel electrophoresis (2DGE) at variable pH ranges. Replicate 2DGE from control and treated cells were scanned and analyzed using Progenesis software. Several of the expressed proteins with a ± 2 fold variation were identified using peptide mass finger printing- MALDI-TOF MS. Notable among the up-regulated proteins at 50-400 ppb DBAN was the proteasomal C-5 component [AA56702]. Identification of this protein was further validated by LC/MS/MS. Up-regulation of proteasomal C-5 protein indicates cellular stress to overcome carbonylated protein accumulation. In conclusion, increased accumulation of carbonylated protein following DBAN treatment may have a role in ND/aging. Ongoing studies using mice at various age levels will further clarify the role of DBP in fetal basis of ND/aging.

215 AGE-RELATED ALTERATIONS IN KAINIC ACID-INDUCED NEURONAL OXIDATIVE DAMAGE.

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Aging is associated with processes that lead over time to an increase in vulnerability to cellular and tissue damage and the probability of death. Recent research findings in brain have highlighted increased excitatory stimulation as contributors to aging as well as neurodegeneration that accompanies Alzheimer's disease, HIV-associated dementia, ischemic stroke, and some forms of epilepsy and cerebral palsy. Associative data from patients' tissue and animal models have widely supported the hypothesis that neuronal oxidative damage is a major effector contributing to neurodegeneration. Mice exposed to kainic acid (KA, 1 nmol/5 µl, icv) showed increase in cerebral F_2-isoprostanes (F_2-IsoPs, 158%), F_4-neuroprostanes (F_4-NeuroPs, 237%) and citrulline (249%) formation 30 min after the exposure. At the same time, pyramidal neurons in the hippocampus of young and old mice had significant reduction in dendritic lenght (60%) and spine density (40%) compared to control (100%). Pretreatment with ibuprofen (14 µg/ml of drinking water for 2 weeks) or α-tocopherol (100 mg/kg, i.p. for 3 days) completely blocked the icv KA-induced increase in cerebral F_2-IsoPs, F_4-NeuroPs, citrulline and decrease in spine density of hippocampal pyramidal neurons in young mice. Importantly, the neuroprotectant effects of these agents were lost in old mice following excitotoxicity. These data strongly suggest that different mechanisms are involved in cerebral neuroprotection of aged mice compared to young mice. The existence and nature of changes with aging have important clinical implications for therapeutic strategies for neuroprophylaxis in both normal aging and neurodegenerative disease. (Supported by ADRC, P50 AG05136 to D.M.).

216 PROTECTIVE EFFECTS OF TAURINE AGAINST REACTIVE OXYGEN SPECIES IN ARPE-19 CELLS.

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Reactive oxygen species (ROS) are generated in the retina as a result of its constant exposure to light radiation. Damaged retinal cells may result in cell death without replacement. Loss of retinal cells is a factor in many ocular diseases. Retinal pigmented epithelial cells (RPE) are responsible for supporting the rods and cones during oxidative stress and the loss of RPE cell functions have been shown to be an early indicator of retinal disease such as age-related macular degeneration. Human RPE cells contain high concentrations of a number of antioxidants including taurine (B-amino-ethane-sulfonic acid), a non-essential amino acid. Deficiency in taurine can lead to loss of RPE cells and blindness. A human RPE cell line (ARPE-19) is used in this present study to model the effects of taurine on oxidative damage. The oxidative stress was provided by ROS such as hydrogen peroxide (H2O2) and menadione. ARPE-19 cells were routinely grown in DMEM/F12 medium plus 10% FBS and gentamicin prior to being switched to a serum free medium since FBS naturally contains taurine. Cells were seeded in 24 well plates and incubated with or without 2mM taurine for 72 hours. The cell cultures were then exposed to various concentrations of H2O2 for 30 minutes. After rinsing, 10% alamarBlue in culture medium was added to each well and the fluorescence developed in 4 hours was measured in a fluorescent plate reader. The results showed that at high cell densities, the LD50 of H2O2 cytotoxicity in ARPE-19 cells was raised from 7.8mM to 27.9mM in taurine treated cells. At low cell density, the LD50 was raised from 7.3mM to 65.8mM. Similar studies are being conducted with menadione cytotoxicity. We demonstrated that taurine, in-vitro, exhibits a partial protective effect against oxidative damage in ARPE-19 cells.

217 INDUCTION OF INFLAMMATORY MEDIATORS AND ANTIOXIDANTS FOLLOWING EXPOSURE OF MACROPHAGES TO PARAQUAT.

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Paraquat (1, 1'-dimethyl-4, 4'-bipyridinium), a commercially important herbicide, is known to be a potent inducer of lung fibrosis in rodents and humans. It is also cytotoxic for nigral dopaminergic neurons and may be an environmental risk factor for Parkinson's disease. Macrophages are phagocytic cells known to release a variety of inflammatory mediators and are thought to participate in the cytotoxicity of paraquat. In many cell types including macrophages, paraquat undergoes redox cycling which can generate active radical species which cause modifications in cellular macromolecules and promotes lipid peroxidation. In the present studies we determined if paraquat activated macrophages to produce inflammatory mediators. Using RAW264.7 murine macrophages, we found that paraquat readily undergoes redox cycling as determined by the formation of hydroxyl radicals as measured by the hydroxylation of terephthalate. This was associated with a time- and concentration-dependent induction of heme oxygenase-1 (HO-1), a potent antioxidant. Following paraquat treatment macrophages were found to express cyclooxygenase-2 (COX-2), inducible nitric oxide synthase and tumor necrosis factor-α mRNA as determined by real time PCR. Maximal induction of HO-1 as well as production of inflammatory mediator gene products was observed after 24 hr and 300 µM paraquat suggesting that they are coordinately regulated. Our data demonstrate

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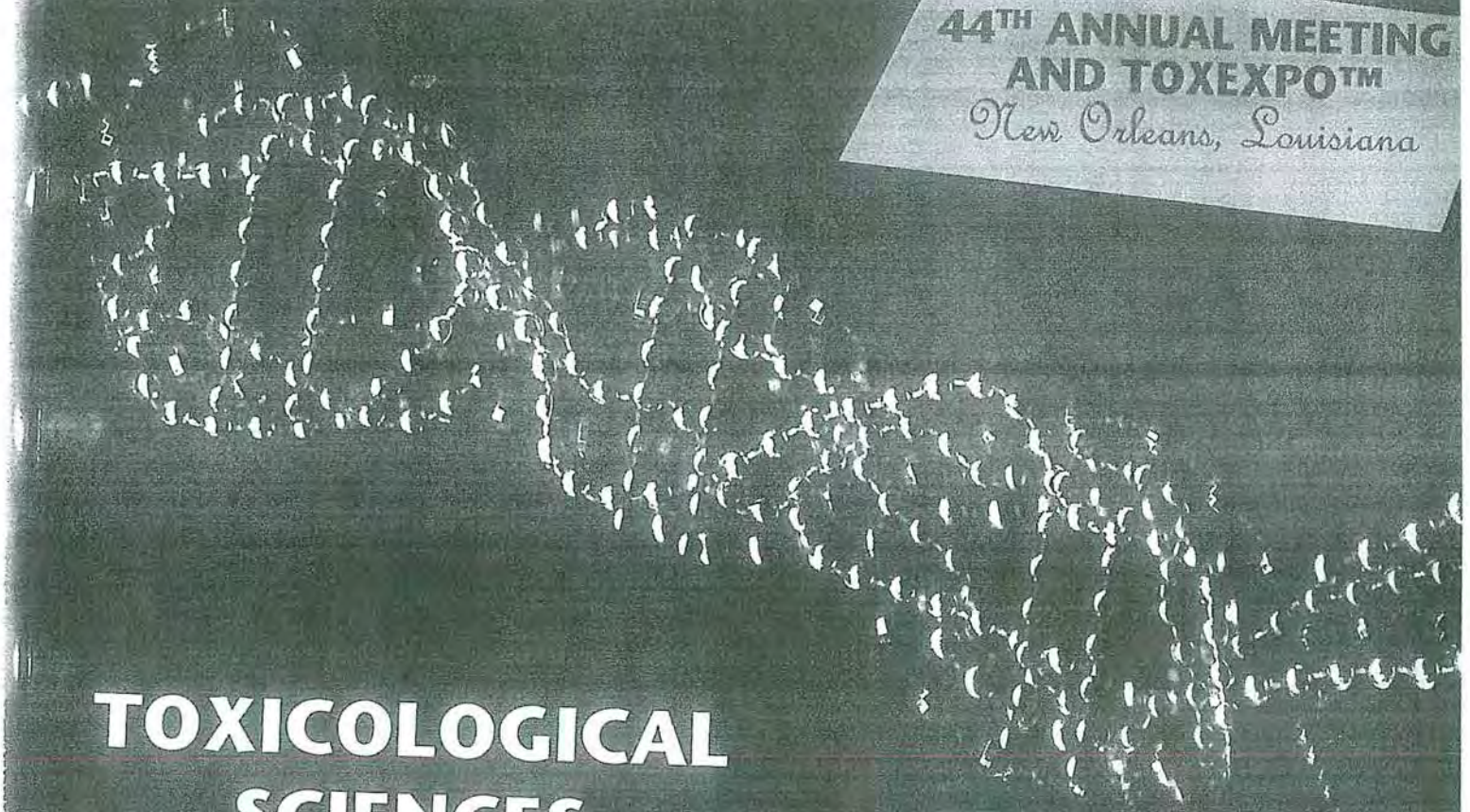

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