1187 AN ELECTROPHILE RESPONSE ELEMENT (EpRE) MEDIATES INDUCTION OF THE \( \gamma \)-GLUTAMYL-CYSTEINE SYNTHETASE REGULATORY SUBUNIT (GCS\(_{\text{R}}\)) GENE.

\( \gamma \)-Glutamylcysteine synthase, the rate-limiting enzyme in glutathione synthesis, consists of a catalytic (GCS\(_{\gamma}\)) and a regulatory (GCS\(_{\text{R}}\)) subunit. Two cis-elements, an AP-1 site (−340−334) and an Electrophile Response Element (EpRE) (−301−290), have been implicated in the regulation of GCS\(_{\text{R}}\) expression in response to pro-oxidants. The AP-1 element has been shown to regulate the basal expression of GCSI gene. However, regulation of GCSI inducible expression is complex (see Moiova and Mulcahy. (1998) JBC 273:14683−14689 and Galloway and McLellan. (1998) Biochem Biophys 376:535−539). The function of the EpRE in GCSI inducibility was further investigated by transient transfection of mutant and synthetic constructs. Mutation of the AP-1 site results in reduced basal expression, while mutation of both the AP-1 and EpRE sites leads to the elimination of induction by both tert-butyldihydroquinone (tBHQ) and b-naphthoflavone (b-NF), confirming our earlier report. While deletion of the 3′ sequences flanking the EpRE reduces both basal and inducible expression, retention of the core EpRE sequence still supports induction by tBHQ. Further analysis of potential cis-elements near the EpRE and AP-1 site is currently under investigation. Nevertheless, current studies support a functional role for the EpRE in mediating inducible gene expression by tBHQ and b-NF. (Funded by grants ES09749 and CA57549).

1188 MOUSE GLUTATHIONE CYSTEINE LIGASE REGULATORY SUBUNIT: GENE STRUCTURE AND REGULATION BY OXIDATIVE STRESS
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The abundant cellular thiol, glutathione (GSH), maintains cellular redox homeostasis. Glutamate is first ligated to cysteine to form \( \gamma \)-glutamylcysteine by glutamate-cysteine ligase (GCL). The first and rate-limiting step in de novo GSH synthesis, GCL is an heterodimer composed of a 70-kD structural subunit and ~150-kD electrophile receptor (EpR) for KB element and CA57549.) found to be a consistent feature of y-glutamylcysteine in vitro. Toxicology Lab/Am University, College Station, TX. Five (funded 3-fold have increased expression. The SUBUNIT (GCS\(_{\text{SI}}\)) GENE, (5 to 0.3 kb) show high basal and 2-fold-inducible activity in rats subjected to tBHQ and b-NF.

EpRE is located near the proximal 3′UTR site. Site-directed mutagenesis demonstrated the requirement of the EpRE for induction. These data suggest that the EpRE and AP-1 site are important in the regulation of GCSI expression. The EpRE and AP-1 site are not required for basal expression. In addition, the EpRE is required for induction by tBHQ. The EpRE is required for induction by tBHQ. The functional role of the EpRE in GCSI inducibility was further investigated by transient transfection of mutant and synthetic constructs. Mutation of the AP-1 site results in reduced basal expression, while mutation of both the AP-1 and EpRE sites leads to the elimination of induction by both tert-butyldihydroquinone (tBHQ) and b-naphthoflavone (b-NF), confirming our earlier report. While deletion of the 3′ sequences flanking the EpRE reduces both basal and inducible expression, retention of the core EpRE sequence still supports induction by tBHQ. Further analysis of potential cis-elements near the EpRE and AP-1 site is currently under investigation. Nevertheless, current studies support a functional role for the EpRE in mediating inducible gene expression by tBHQ and b-NF. (Funded by grants ES09749 and CA57549).

1189 GENE EXPRESSION ANALYSIS OF MOUSE LIVER AND MOUSE HEPATOCYTES FOLLOWING EXPOSURE TO OXIDATIVE STRESS AGENTS MENADIONE AND DIQUAT.

Transcription profiling following compound exposure may be predictive for specific toxicity processes. Changes in gene expression were determined in livers from CD-1 mice given single (c.e.) dose treatments of menadione (40 mg/kg) or diquat dihydroxide (50 mg/kg), two mechanically related oxidative stress compounds. Animals were killed at 6,12, 24 and 48h following dosing. In addition, primary cultures of hepatocytes from untreated mice were exposed to 40μM menadione or 50μM diquat for 6 and 24h in vitro. The pattern of gene expression following treatment groups were classified as up-regulated, down-regulated, or unchanged. Affymetrix GeneChip analysis was used to monitor the relative abundance of approximately 6,500 murine genes and ESTs. An approximately 3% to 6% of sequences on the GeneChip exhibited 2-fold or greater change in expression in treated groups compared to controls for both in vivo and in vitro studies. 32-45% and 24-31% of sequences showing 2 fold or greater change in expression were common to both chemical treatments in vivo after 6h and 24h respectively. Similarly, 32-51% and 13-37% of genes showing 2 fold or greater change in expression were common to both chemical treatments in vitro after 6h and 24h respectively. These results suggest a substantial percentage of genes are similarly affected following exposure to two related redox cyclers. Up-regulation of acute phase proteins and down-regulation of metabolic enzymes were found to be a consistent feature of in vivo toxicity. Analysis of in vitro samples revealed a markedly different pattern of gene expression, although there was a high concordance between the two oxidative stress compounds. This partly reflects differences in complexities of toxic responses in vivo versus in vitro.

1190 1,2-DICHLOROBENZENE: ACTIVATES THE NUCLEAR TRANSLOCATION OF ACTIVATOR PROTEIN-1, NUCLEAR FACTOR-κ B AND ELECTROPHILE RESIDENT ELEMENT IN HEPATOCYTES ISOLATED FROM MALE FISCHER 344 RATS.
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1,2-Dichlorobenzene (1,2-DCB), an industrial solvent, is a known hepatotoxicant. Work done by our group and others suggests that two oxidative events that mediate liver injury occur following 1,2-DCB treatment. The first oxidative phase is caused by the bioactivation of 1,2-DCB to reactive intermediates, while activation of inflammatory cells (i.e. Kupffer cells) mediates a second oxidative stress. To investigate the molecular and cellular basis of hepatic toxicant oxidative stress, hepatocytes were isolated from male Fischer 344 rats, and examined for glutathione disulfide (GSSG), a marker of oxidative stress, and enhanced nuclear translocation of activator protein-1 (AP-1), nuclear factor κB (NF-κB) and electrophile responsive element (EpRE) transcription factors following incubation with 1,2-DCB (3.55 or 7.12 nmol).

Oxidative stress occurred in hepatocytes incubated with 1,2-DCB as concentrations of GSSG were maximally elevated (15% above control) by 1 hr, while glutathione concentrations progressively decreased to 78% of control by 6 hr. The activities of AP-1 and NF-κB were increased by as much as 3-fold by 6 hr of 1,2-DCB treatment as compared to control. Nuclear translocation of EpRE was also enhanced by 3-fold but occurred earlier (2 hr) following 1,2-DCB treatment. Moreover, jun N-terminal kinase was activated in 1,2-DCB treated hepatocytes by 0.5 hr and was maintained for up to 2 hr. These data suggest that 1,2-DCB induced oxidative stress triggers a cascade of molecular processes that promote the nuclear translocation of transcription factors involved in regulating the expression of oxidative sensitive genes in hepatocytes. (Supported by Center Grant P50-1806694 and an AFPE Fellowship.)

1191 OXIDATIVE INJURY MODULATES EXTRACELLULAR MATRIX-REGULATED NF-Κ B BINDING ACTIVITY IN VASCULAR SMOOTH MUSCLE CELLS.
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Repeated exposure of rats to allylamine induces proliferative (i.e. atherogenic) phenotype in vascular smooth muscle cells (vSMCs). This atherogenic response is mediated by permanent changes in signaling pathways associated with integrin-mediated extracellular matrix interactions. The present study investigated the relationship between increased proliferative capacity and extracellular matrix signaling in vSMCs derived from rats subjected to an atherogenic allylamine regimen. Because extracellular matrix signaling is coupled to integrin-mediated activation of NF-κB binding, electrophoretic mobility shift assays and measurements of cell proliferation were conducted to compare cells seeded on plastic, collagen, fibronectin, and laminin. Allylamine cells displayed a proliferative advantage when seeded on plastic, fibronectin and laminin, but not when seeded on collagen. Five major NF-κB complexes were reduced by 7-25% in the non-dense and cell-free processes of collagens. The predominance of individual complexes was substrate-dependent with complexes 2, 3, 4 and 5 being prominently induced on plastic, laminin and fibronectin, and complexes 3 and 4 on collagen. Allylamine cells exhibited increased NF-κB binding relative to controls when seeded on plastic, laminin and fibronectin, but not on collagen, indicating that NF-κB binding activity correlates with the occurrence of proliferative phenotypes in vSMCs. These results implicate integrin-coupled NF-κB signaling in the induction of atherogen
genic phenotypes by allylamine. (Supported in part by NIH Grant ES09160 and a TAMU Interdisciplinary Grant.)

1192 ACTIVATION OF HEPATIC NF-κB BY POLYCHLORINATED BIPHENYLS (PCBs) IN VIVO AND IN CULTURED RAT HEPATOCYTES.


Polychlorinated biphenyls (PCBs) are environmental pollutants that, due to their persistence and biomagnification, raise concerns about the health consequences of long-term exposure. The mechanism of the promoting activity of PCBs has not yet been determined. Previous studies show that oxidative stress occurs during metabolism of PCBs, with the formation of free radicals and oxidative DNA damage, which may contribute to their promoting activity. In this study, we examined whether oxidative stress-sensitive transcription factors NF-κB or AP-1 would be induced by PCBs in vivo or in primary hepatocyte culture. Male Sprague-Dawley rats were injected i.p. with corn oil, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153, 30, 150 or 300 μmol/kg), 3,3',4,4'-tetrachloro-biphenyl (PCB-77, 30, 150 or 300 μmol/kg) or both PCBs (30 or 150 μmol/kg each). Rats were killed 2, 6, or 24 hours, or 2, 6, and 10 days after the PCB injection. Electrophoretic mobility shift assays were performed to determine the DNA binding activities of NF-κB and AP-1. The highest NF-κB DNA binding activity was observed in rats receiving higher doses of PCB-153 (150 and 300 μmol/kg), with a peak occurring 2 days after injection. NF-κB activity was also increased in rats receiving both PCBs to a lesser extent, but no effect was seen in rats treated with PCB-77. Primary rat hepatocytes were cultured on collagen gels in serum-free L-15 medium with or without PCBs. NF-κB binding activity in hepatocytes culture was increased after a 48 hour exposure to PCB-153 (20 μM). These results show that hepatic NF-κB binding activity can be activated by specific PCBs in vivo and in cultured rat hepatocyte culture, indicating hepatic oxidative stress induced by PCBs. (Supported by ES 07380.)

1193 BCL-XI AND BCL-2 EXPRESSION IN RAT VESTIBULAR AND AUDITORY BRAINSTEM NUCLEI FOLLOWING IN VIVO EXPOSURE TO m-DINITROBENZENE.

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m-Dinitrobenzene (DNB) induces dose-dependent givascular lesions in auditory and vestibular brainstem nuclei with secondary damage to neurons. This study investigates Bcl-2 and Bcl-x expression in rat brainstem nuclei during DNB-induced neurotoxicity in vivo. Male Fisher 344 rats were given either 7 mg/kg (mild lesion) or 10 mg/kg (severe lesion) DNB in DMSO twice daily via intraperitoneal injection for 1-2 days and sacrificed at 24, 48, 54 and 72 h after the first dose. Immunohistochemistry and western immunoblotting were used to examine expression of Bcl-X and Bcl-2 in affected regions such as inferior colliculi, deep cerebellar roof, vestibular, and cochlear nuclei. Regions resistant to DNB-induced neurotoxicity, such as cerebellum and hippocampus, were also examined. Positive staining for Bcl-x and Bcl-2 was noted in neuronal somata, dendrites and proximal axons of brainstem neurons in rats. The mRNA level of Bcl-x and Bcl-2 in the inferior colliculus, deep cerebellar roof and cochlear nuclei of all control and DNB-exposed animals. A time-dependent increase in Bcl-x and Bcl-2 expression was observed in cerebellum and hippocampus in animals exposed to 10 mg/kg DNB, but not in controls at any timepoint. DNB-related induction of both proteins began at 24 h and was maximal at 72 h. Increased levels of Bcl-x and Bcl-2 were observed in immunohistochemical analysis in all brainstem nuclei examined from animals exposed to 7 mg/kg DNB twice daily for three days. No expression of Bcl-x and Bcl-2 was observed in neurons and astrocytes of control brains. Western blotting revealed moderate basal expression of Bcl-x and Bcl-2 in the inferior colliculi, deep cerebellar roof and cochlear nuclei of all control and DNB-exposed animals. A time-dependent increase in Bcl-x and Bcl-2 expression was observed in cerebellum and hippocampus in animals exposed to 10 mg/kg DNB, but not in controls at any timepoint. DNB-related induction of both proteins began at 24 h and was maximal at 72 h. Increased levels of Bcl-x and Bcl-2 were observed in immunohistochemical analysis in all brainstem nuclei examined from animals exposed to 7 mg/kg DNB twice daily for three days. No increase in expression of Bcl-x and Bcl-2 was observed by western blot analysis with the 7 mg/kg DNB dosing regimen. These results suggest that differential expression of Bcl-x and Bcl-2 correlates with lack of susceptibility to DNB neurotoxicity. (This research is supported by PHS·NIH Grants ES08846 & ES06103.)

1194 DIFFERENTIAL EXPRESSION AND ACTIVITY OF Nrf2 IN LUNGS OF HYPEROXIA-SUSCEPTIBLE AND -RESISTANT MICE.


Acute respiratory distress syndrome (ARDS) is a major lung disease mediated by reactive oxygen species. An in vivo model of acute lung injury with similar features of ARDS has been produced by administration of hyperoxia (>95% oxygen) to animals. Among inbred mouse strains, C57BL/6J (B6) have greater sensitivity to hyperoxia than other inbred strains, including C3H/HeJ (C3). We recently identified significant hyperoxia-susceptibility quantitative trait locus (QTL) on mouse chromosome 2. A strong candidate gene within this QTL is Nrf2, an essential transcriptional regulator of antioxidant enzymes that play key roles in protecting cells against carcinogenicity and oxidative stress. To test the hypothesis that Nrf2 confers differential susceptibility to oxygen toxicity, Nrf2 mRNA expression (by reverse transcription-polymerase chain reaction) and DNA binding activity (by gel shift/super-shift analyses) were evaluated in the lungs of C3 and B6 mice exposed to hyperoxia. Exposure markedly and similarly induced Nrf2 mRNA expression at 90 min and 6 hr in both strains, compared to strain-matched air-controls. The mRNA levels returned to basal levels at 24 hr in both strains. A second increase in Nrf2 mRNA expression occurred after 48 hr in C3 mice; in B6 mice, the mRNA level did not increase again until after 72 hr. The basal Nrf2 activity was greater in B6 mice compared to C3. After 90 min exposure, Nrf-2 activity increased in C3 mice proportional to gene expression and remained elevated until 48 hr. In contrast, Nrf-2 activity was markedly decreased in B6 mice by 6 hr and continued to decrease during the exposure. These results indicate that there is a significant strain-specific variation in Nrf2 expression and activity after hyperoxia exposure. The results also suggest the early increase in Nrf-2 activity conferred greater protection against oxidative injury in resistant C3 mice, while decreased Nrf-2 activity may enhance susceptibility in B6 mice. (Support: ES 09606, HL 57142.)

1195 ACTIVATION OF ACTIVATOR PROTEIN-1 BY REACTIVE OXYGEN SPECIES ASSOCIATED WITH ASBESTOS.

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Inhalation of asbestos causes alterations in cell signaling cascades, gene expression, cell injury and cell proliferation which may lead to pulmonary fibrosis, lung cancer, or mesothelioma. Asbestos-mediated free radical reactions are believed to trigger a number of cellular and molecular events that may promote fibrogenesis and carcinogenesis. Because activator protein 1 (AP-1) plays an important role in pre-neoplastic-to-neoplastic transformation, tumor promotion and metastasis, we studied the possible activation of AP-1 in vivo in cultured J66 cells and in vivo using transgenic mice after exposure to crocidolite asbestos. In vitro exposure to asbestos, caused a dose- and time-dependent activation of AP-1 in cultured J66 cells. Exposure of mice to crocidolite asbestos caused a significant (22-fold) activation of AP-1 in bronchiolar tissue compared to a mere 10-fold increase in the lung tissue. The induction of AP-1 in asbestos exposure appears to be mediated through the phosphorylation of mitogen-activated protein kinases, Erk 1 and Erk 2. Hydroxy radical scavengers inhibited asbestos-induced AP-1 activation. These data support the hypothesis that oxygen radical mechanisms may be associated with pulmonary fibrosis and carcinogenesis.

1196 METHODS FOR MEASURING EXPRESSION OF IL-1 ALPHA, NITRIC OXIDE SYNTHASE, AND NITRIC OXIDE IN F-344 RAT SKIN IN RESPONSE TO DERMAL EXPOSURES TO FUELS OR SOLVENTS.

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Organic chemicals such as jet fuels and solvents are recognized to cause skin irritation after dermal exposures. The molecular responses to these chemicals that result in acute irritation are not understood well enough to allow the establishment and choice of safe exposure limits. We conducted studies to determine the feasibility of measuring various inflammatory factors, including IL-1 alpha, nitric oxide synthase, and nitric oxide. Male F-344 rats were exposed to organic chemicals using the Hill Top Chamber®. Three hours after the exposures treated and control skin samples (1 to 1.5g) were collected. Light microscopy evaluation was performed on formalin-fixed and