

[Ramsay, AJRCCM 164:155, 2001]. Oxidative modifications of CC10, indicated by reactivities with 2, 4-dinitrophenylhydrazine (DNPH), appear to account for some of the decreases in TAF concentrations of CC10. The present studies were to test the hypothesis that hyperoxia also exerts pretranslational effects that may contribute to the diminished CC10 levels. Adult male ICR mice were placed in greater than 95 percent O₂ for up to 96 h. Increases in lung weight-to-body weight ratios were not observed through 72 h of hyperoxia, thus indicating minimal lung injury through this duration of exposure. Levels of CC10 mRNA were assessed by real time PCR, with 18S as reference. By 48 h of hyperoxia, lung levels of CC10 mRNA decreased to less than half of the levels observed in air-breathing control animals, and by 96 h, CC10 message levels were less than 5 percent of controls. In contrast, message levels of glutathione reductase in the lungs of animals in hyperoxia increased through 72 h to levels five times the levels in air-breathing control animals, then declined sharply between 72 and 96 h of hyperoxia. Lung GR activities were not increased in animals exposed to hyperoxia. The data indicate that hyperoxia can exert pretranslational effects that may contribute to the diminished levels of CC10 that we observed in premature human infants. The marked differences between CC10 and GR mRNA responses indicate gene-specific effects and/or selective and early damage to the Clara cells in the lungs of animals and possibly in humans exposed to hyperoxia. Supported in part by GM44263.

51 HYPEROXIC LUNG INJURY IN MICE WITH GENETIC DEFICIENCIES IN GLUTATHIONE REDUCTASE ACTIVITIES.

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Glutathione-dependent mechanisms are vital to antioxidant defense mechanisms, and we have demonstrated previously that transgene-driven enhancement of glutathione reductase (GR) activities can increase dramatically cell resistance to oxidant toxicities *in vitro*. Conversely, enhanced sensitivities to oxidants are observed in cells with attenuated GR activities from antisense expression of the GR transgene. The toxicological relevance of GR *in vivo* is attributed to the effects on toxicities of BCNU, which inhibits GR *in vitro* and *in vivo*, but the specificity of BCNU is less than is often assumed. In the present studies, we investigated the susceptibilities to hyperoxic lung injury of a line of mice that exhibit GR activities in livers and lungs that are less than 10% of the respective activities observed in the parent strain (C3H) of mice. The founder mouse of this GR hypomorph strain was generated by administration of isopropyl methanesulfonate, and the trait bred to homozygosity (Pretsch, Genet Res Camb 73:1, 1999). The GR hypomorph animals do not exhibit any remarkable phenotype, and the trait has remained stable for over 20 generations. We obtained breeding pairs and re-derived progeny at our institution. Lung and liver GR activities in the GR hypomorph mice were 6 and 9% of control C3H mice, whereas respective glutathione peroxidase activities and GSH levels were not different. Adult male mice of the GR hypomorph strain (Gr1a1Neu) exposed to greater than 95 percent O₂ exhibited increases in lung weight to body weight ratios by 72 h of exposure, whereas increased lung weights in the wild type C3H mice were observed only after 96 h of continuous exposure to hyperoxia. The greater susceptibility of the Neu than of the C3H mice to hyperoxia is consistent with significant contributions of GR to antioxidant functions, but the modest effects on injury of such marked differences in GR activities suggest compensatory responses. Supported in part by GM44263.

52 EXPRESSION AND LOCALIZATION OF P70 ALBUMIN PRECURSOR PROTEIN AND PHI AP3 IN OXIDATIVELY STRESSED VASCULAR SMOOTH MUSCLE CELLS.

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Redox signaling by benzo(a)pyrene (BaP) in vascular smooth muscle cells (vSMCs) involves activation of protein binding to a DNA regulatory sequence termed the antioxidant response element (ARE). ARE sequences (RTGAYNNNGCR) are similar to consensus TPA-response elements (RTGACTCA), cyclic AMP response elements (TGACGTCA) and Maf-response elements (TGCTGAGTCA), evidence of functional overlap in redox signaling among multiple cellular pathways. Recently, this laboratory identified p70 albumin precursor protein (APP) and Phi AP3 as novel components of the redox sensing machinery in vSMCs. The present studies were conducted to characterize the expression and cellular localization of these proteins in BaP-treated cells. Affinity-purified IgG was generated against APP and Phi AP3 peptide antigens. Two immune-reactive bands of Mr of 35- and 70-kDa were detected for APP in extracts of the cytosolic and nuclear compartments of vSMCs. Short-term oxidant treatment (0.5 hr) selectively increased p70 expression in the cytosolic compartment, while the p70 immune-reactive protein accumulated in the nucleus within 0.5 - 1 hr, along with a concomitant increase in p35 immunoreactivity. Anti-Phi AP3 immunoreactivity was detected at Mr 18-, 40 and 65-kDa in

the nuclear fraction, while Mr 18- and 40- and 42-kDa proteins were predominant in the cytosolic fraction. Treatment of vSMCs with 3uM BaP abolished the nuclear p65 Phi AP3 signal and enhanced the appearance of p40 immunoreactivity. Fluorescence microscopy of BaP-treated cells showed diffuse Phi AP3 cytosolic staining, while APP immunofluorescence was confined to the nucleus. These results suggest that BaP alters the expression and localization of APP and Phi AP3, and implicate these proteins in redox signaling in vSMCs. (Supported by NIH Grants ES04849, ES04917, and ES09106).

53 NF-KB DYSREGULATION IN ATHEROSCLEROTIC VASCULAR SMOOTH MUSCLE CELLS: COMPLEX COMPOSITION AND REDOX SENSITIVITY.

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Repeated exposure to chemical oxidants leads to induction of atherogenic vascular smooth muscle cell (vSMC) phenotypes. These cells are characterized by heightened proliferative activity, altered integrin expression, and upregulated NF- κ B activity. NF- κ B, a dimer of Rel proteins, is a transcription factor typically located in the cytoplasm *via* association with an inhibitory protein I κ B. After phosphorylation by the IKK complex, I κ B is ubiquitinated and destroyed, allowing NF- κ B dimers to translocate to the nucleus and effect transcriptional regulation of a variety of gene targets, including osteopontin, a cytokine implicated in the maintenance of atherogenic vSMC phenotypes. In the present studies, male Sprague-Dawley rats (175-200g) were gavaged once daily for 20 consecutive days with 70 mg/kg allylamine, a vascular specific pro-oxidant. Aortic smooth muscle cells were isolated by enzymatic digestion and maintained in serial culture. Cells were seeded at 100 cells/mm², growth arrested for 72 hours, and then released into growth by addition of serum mitogens. Immunoprecipitated IKK α was phosphorylated to a greater extent in allylamine cultures than controls, indicating a higher level of NF- κ B activation. Rel A/p65 proteins were phosphorylated to a lesser extent in allylamine cultures, suggesting changes in the ability of this protein to regulate gene induction. The levels of other NF- κ B constituent proteins in nuclear extracts were altered, with p52 levels increased and RelB levels decreased, in the nuclei of allylamine cells relative to controls. Gel shift analysis using nuclear proteins from control and allylamine cultures treated with PDTC or N-acetyl cysteine showed that NF- κ B activity in allylamine cells was more sensitive to antioxidant inhibition than respective controls. These results implicate altered redox homeostasis as a key regulator of NF- κ B activation in chemical atherogenesis. (This work was supported in part by NIH grants HL62539 and ES09016).

54 ROLE OF BIP/GRP78 IN 11-DEOXY-16, 16-DIMETHYL PROSTAGLANDIN E2 MEDIATED CYTOPROTECTION IN RENAL EPITHELIAL CELLS.

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Treatment with 11-Deoxy-16, 16-dimethyl prostaglandin E₂ (DDM-PGE₂), a stable synthetic analog of PGE₂ protects renal proximal tubule epithelial cells (LLC-PK₁) against oncotic/necrotic cell death induced by hydrogen peroxide (H₂O₂), iodoacetamide (IDAM), and 2, 3, 5-tris-(glutathion-S-yl)hydroquinone (TGHQ), but not against cisplatin or mercuric chloride induced apoptotic cell death. Utilizing mass spectral and western blot analyses we showed that cytoprotection was associated with the up-regulation of at least six proteins, including the major endoplasmic reticulum (ER) chaperone, glucose-regulated protein 78 (Bip/Grp78). To elucidate the role of Grp78 in cell injury, we investigated cytoprotection using LLC-PK₁ cells in which induction of *grp78* expression was disrupted by stable expression of an antisense *grp78* RNA (pkAS*grp78*). As anticipated, DDM-PGE₂ failed to induce Grp78 in pkAS*grp78* cells, with a concomitant inability to provide cytoprotection against TGHQ, H₂O₂, or IDAM. In contrast, DDM-PGE₂ induced Grp78 and afforded cytoprotection against all three toxicants in cells transfected with empty vector (pkNEO). These data suggest Grp78 plays an important role in DDM-PGE₂ mediated cytoprotection. Furthermore, using 2D gel electrophoresis coupled with MALDI-TOF peptide mass mapping and post source decay, we compared the pattern of protein induction in pkAS*grp78* and pkNEO cells following DDM-PGE₂ pretreatment. Our results revealed that DDM-PGE₂ induced several proteins in pkNEO cells, but not in pkAS*grp78* cells, including S100 calcium binding protein A2, proteasome subunit C2, galectin-1, galectin-3, myosin light chain, and molecular chaperones such as heat shock protein 27 and chaperonin 10. The findings suggest that these proteins may act in concert with Grp78 during DDM-PGE₂ mediated cytoprotection against oncotic/necrotic cell death. (GM56321, ES07784).